GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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CORRIGENDA

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Page 422, line 3 from bottom, for "the following F_2 ratio" read "the following ratio."

Page 425, line 6, for "C. N. COLLINS" read "G. N. COLLINS."

Page 428, line 10, for "was found to 41.5" read "was found to be 41.5."

Page 430, line 2, for "17 plants" read "17 virescent plants."

Page 451, line 10 from bottom, for "figures la and lb" read "figures 1a and 1b."

Page 457, line 3 from bottom, for "(PP)" read "(pp)."

Page 459, line 4, for "none were long" read "none of the 4 were long."

Page 504, line 12, for "Bibliographica" read "Bibliographia."

Page 544, line 17 from bottom, for "octahybrid" read "octohybrid."

Page 558, TABLE 31, line 3, for "1 2 6 4 5 6 n^1" read "1 2 3 4 5 6 n^1."

Page 563, line 11, for "these formula" read "these formulae."

Page 580, line 2 of Part II, for "successive" read "recessive."

Page 581, line 6, the denominator of the second term of the equation, for $\frac{}{2!\,m-2!}$ read $\frac{}{2!\,m-2!\,n^4}$

Page 589, line 3, for "MILLER" read "MULLER."

Page 591, line 3 from bottom, for "var. *Dunlap*" read "var. Dunlap."

Page 597, line 4, for "octaploid" read "octoploid."

Page 602, line 14, for "expèrimentale" read "expérimentale."

Page 603, line 20, for "Viola-arten" read "Viola-Arten."

Page 606, line 5, from bottom, for "Na Plus Ultra" read "Ne Plus Ultra."

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Page 48, line 17, for "NORTHOP" read "NORTHROP."

Page 60, lines 16 and 24, for "VELENOVSKY" read "VELONOVSKY."

Page 82, LITERATURE CITED, for "BARANOW" read "BARANOV."

Page 83, line 45, for "VELENOVSKY" read "VELONOVSKY."

Page 83, LITERATURE CITED, for "TISCHLER, J." read "TISCHLER, G."

Page 87, lines 3, 8 and 15, for "heterotypic" read "homotypic."

Page 122, line 5, for "The F_1 seed" read "The F_2 seed."

Page 122, last line, for "then" read "than."

Page 165, LITERATURE CITED, for "ROMASHOV" read "ROMASHOFF."

Page 254, lines 21 and 22, for "EVERMAN" read "EVERMANN."

Page 270, line 8, for "heterozyous" read "heterozygous."

Page 277, footnote, line 1, for "ISHIMARA" read "ISHIWARA."
Page 278, LITERATURE CITED, for "EVERMAN" read "EVERMANN."
Page 279, LITERATURE CITED, for "SACH" read "SACHS."
Page 283, Table 5a, for "PEDIGREE STIPPLED STIPPLED NON-STIPPLED" read "PEDIGREE SCHOOL STIPPLED NON-STIPPLED."
Page 294, LITERATURE CITED, for "SINNOT" read "SINNOTT."
Page 309, line 8, for "*F. vesca* Rostrup" read "*F. vesca rosea* Rostrup."
Page 343, line 2, for "tomozygous read "homozygous."
Page 343, line 3, for "hnk-eyed" read "pink-eyed."
Page 389, line 23, for "chromosome" read "autosome."

L. Bataillon

WILLIAM BATESON

(FRONTISPIECE)

WILLIAM BATESON was born in Whitby, Yorkshire, England, August 8, 1861, and died at Merton, Surrey, February 8, 1926. He entered RUGBY SCHOOL in 1875, and in 1878 went to ST. JOHNS COLLEGE, Cambridge, of which his father was at the time Master. He took first class in the Natural Sciences Tripos in 1882 and in 1883 was in the first class in Part II. In 1885 he was elected to a Fellowship in ST. JOHNS COLLEGE; in 1908 he was appointed to the chair of Biology which was especially created for him at Cambridge; in 1910 he resigned his Professorship and accepted the Directorship of the JOHN INNES HORTICULTURAL INSTITUTE near London, which under his guidance became one of the leading stations of the world for the study of genetics.

In the summers of 1883 and 1884 he came to the United States to study Balanoglossus at Hampton, Virginia, where he met W. K. BROOKS, through whom he became interested in questions of variation and heredity.

In 1886, 1887 he traveled in the Central Asian Steppe as for as Turkestan, studying the relation of environmental conditions to variation; and in 1894 he published his well-known book on the "Materials for the study of variation," in which he brought together a large amount of widely scattered information bearing upon discontinuity in variation. Here he developed certain views concerning the relation of species to discontinuity which led to his assuming a skeptical attitude towards DARWIN'S theory of natural selection acting on continuous variations.

In 1896 he was married to Miss BEATRICE DURHAM and in 1898 moved to his house at Grantchester where he established a private experiment station at which numerous breeding experiments were instituted. When in 1900 MENDEL'S paper was brought to light, he immediately recognized its far-reaching significance and in 1902 he published "Mendel's principles of heredity—a defence," and with several collaborators he presented (1902–1908) in a series of "Reports to the Evolution Committee of the Royal Society" an important body of experimental results from comprehensive experiments with stocks, sweetpeas, poultry, Lepidoptera, etc., which confirmed and materially extended the Mendelian principles. His defense of Mendelism and his vigorous attack on those who had tried to minimize its importance went far towards bringing MENDEL'S work to the attention of a wider audience. In 1909 he published his "Mendelian principles", a greatly enlarged second edition of which was published in 1913, summarizing his work and that of the Cambridge school as well as of other workers.

In 1907 he delivered at YALE the Silliman Lectures, which were published in book form in 1913 under the title "Problems in genetics." This work was reprinted in 1916.

He was Chairman of the Zoological Section of the BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE at its Cambridge meeting in 1904; President of the THIRD INTERNATIONAL CONFERENCE ON GENETICS in 1906; and President of the BRITISH ASSOCIATION at its meeting in Australia in 1914. In 1920 he gave the Croonian lecture; and in 1922 delivered an invitation address before the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

at its meeting in Toronto. He also gave the JOSEPH LEIDY Memorial Lecture in Philadelphia in 1922.

In 1911, in collaboration with Professor R. C. PUNNETT, he established the Journal of Genetics. In his inaugural address as President of the THIRD INTERNATIONAL CONFERENCE ON HYBRIDIZATION AND PLANT BREEDING, he proposed the name "genetics" for this branch of biological science, and he has been the author of many of the technical terms now in common use in genetics, for example, factor, allelomorphs, zygote, homozygous, heterozygous, epistatic, hypostatic, coupling, repulsion, etc.

He was elected Fellow of the ROYAL SOCIETY OF LONDON in 1894, was awarded the DARWIN Medal in 1904, and the Royal Medal in 1920; was Fullerian Professor in the ROYAL INSTITUTE 1912-1914, and was elected Trustee of the BRITISH MUSEUM in 1922. He received the degree of Master of Arts *honoris causa* from the University of Sydney and Doctor of Science *honoris causa* from the Universities of Sheffield, England and Perth and Melbourne, Australia. He was also an Honorary Member of many of the Academies of Science and Biological Societies of Europe and America.

In 1922 the Editorial Board of Genetics voted to publish BATESON's portrait as frontispiece to volume 8 of Genetics, but being unable at that time to secure a satisfactory photograph for the purpose, the matter was postponed. The photograph here reproduced was taken at Christmas 1922 and is pronounced by Mrs. BATESON to be, in her judgment, the best available portrait of her husband. We are deeply indebted to Mrs. BATESON for copies of this photograph and also for the biographical data from which this sketch has been in part prepared.

The cost of engraving and printing this portrait has been generously contributed by Doctor JOHN HARVEY KELLOGG, well-known Superintendent of the BATTLE CREEK SANITARIUM, Battle Creek, Michigan, whose interest in eugenics led him to establish, about twenty years ago, a corporation known as the RACE BETTERMENT FOUNDATION, which organized the first RACE BETTERMENT CONGRESS in 1914, and which established in 1915 an EUGENICS REGISTRY. The RACE BETTERMENT FOUNDATION of which Doctor KELLOGG is President has recently established BATTLE CREEK COLLEGE, one of whose major aims is the promotion of eugenics and race betterment ideals.

THE EFFECT OF CHROMOSOME ABERRATIONS ON DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

JU-CHI LI

Columbia University, New York City

Received May 27, 1926

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INTRODUCTION

In the course of the breeding work on Drosophila in the COLUMBIA UNIVERSITY Laboratory, a number of distinct somatic types have arisen that have been interpreted as due to deficiencies, duplications or translocations of parts of chromosomes, and to non-disjunction, resulting in the addition or loss of a whole chromosome. Some of these changes have been shown to be lethal in varying degrees, destroying individuals at specific stages of development. Such lethal effects are recognized mainly from distorted ratios among the surviving adults; and so far little effort has been made to determine the precise stage at which death takes place.

In the present work, the developmental stages of these flies are followed by the use of a new technique, by which eggs, larvae, and pupae could be handled in large numbers. As a detailed description of these methods is given in the appendix, only the most important features of the technique need be brought out here. The stocks used for analysis were first purified by selection and inbreeding with the purpose of making them homozygous. Each test was accompanied by control matings. Both the experimental and the control series were treated alike under identical environmental conditions. Matings were made in 4×1 inch vials; and the flies were raised in a constant temperature incubator (24°C) on standardized food. Eggs were isolated and counted every twenty-four hours. After hatching, a second egg count was taken to determine the number of eggs that hatched into larvae and also the number that died in the egg stage. The counts of the pupae followed; and finally, the adults that emerged from the pupa cases were classified to ascertain which, if any, of the expected classes were missing.

EXPERIMENTAL RESULTS

Notch deficiencies

The first "Notch" mutation was found in 1914 by DEXTER (1914) who showed that the character was sex-linked, dominant in the female and lethal in the male. BRIDGES soon afterward found a second "Notch" and located it at 1.5 units to the right of the white locus (MORGAN and BRIDGES 1916). Later several other Notches were found by MORGAN, BRIDGES, MULLER and GOWEN. One, Notch 6, found by BRIDGES, showed a new characteristic for Notch, namely, "Pseudo-dominance" for facet (METZ and BRIDGES 1917). When the Notch 6 female was crossed to facet males, all of the Notch 6 daughters were at the same time facet. This similarity to the behavior of the forked in the forked-Bar deficiency (BRIDGES 1917) and vermilion in vermilion deficiency (BRIDGES 1919) suggested that Notch 6 is a deficiency that includes the locus for the facet character. Notch 8, found by MOHR in 1918, has been the object of extensive study (MOHR 1919, 1923). Notch 8 showed all the phenomena encountered in the previous Notches, but differed from them in involving a longer section. Thus, Notch 8 gave pseudo-dominance and exaggeration with facet, with white and its nine allelomorphs, located 1.5 units to the left of facet and with Abnormal, located 1.5 units to the right of facet. This interval of approximately 3 units is the minimum measure of the length of the deficient region. Very extensive linkage tests showed that crossing over was entirely eliminated from the deficient region. Up to

the year 1924, twenty-five Notches had been discovered, four of which (N8, N9, N18, and N21) have been shown to be extensive enough to cover the locus for white.

Notch 8 of MOHR, Notch 18 of BRIDGES and two others, Notch 11 and Notch 19 (both found by BRIDGES), have been used by the author in making a comparative study of the differences in the length of the deficient regions. From linkage results with four mutant characters, namely, scute, broad, echinus and ruby ($s_c\ b_r\ e_c\ r_b$) and later with five characters, namely, scute, broad, apricot, echinus and ruby ($s_c\ b_r\ w^a\ e_c\ r_b$) through eight successive generations, the conclusion was reached that the deficiency of Notch 18 is slightly greater than that of Notch 8 and also that in Notch 18 the adjacent regions are reduced somewhat in the amount of crossing over. The extent of deficiency of Notch 19 is probably less than that of Notch 11 and in both Notch 19 and Notch 11 is much less than in Notch 18 and Notch 8. (Data unpublished.)

Notch male

In carrying out the above mentioned linkage experiments the stocks of Notches (N8, N11, N18, and N19) were all crossed to the same stock ($s_c\ b_r\ e_c\ r_b$) for eight generations, and they were then considered satisfactory material for an analysis of the fate of the Notch males, since in a large proportion of their autosomal genes they had presumably become homozygous and homogeneous. A forked stock (pedigree No. 15138) closely inbred for at least eighteen generations was obtained through the kindness

TABLE 1

Notch $8/s_c\ b_r\ w^a\ e_c\ r_b$ ♀ × *forked* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+♀	+♂	N♀
56	18	32.1	38	10	17.8	28	0	0.0	9	9	10
162	59	36.4	103	19	11.7	84	1	0.6	32	17	34
64	15	23.4	49	9	14.0	40	0	0.0	11	17	12
77	21	27.3	56	10	13.0	46	0	0.0	16	15	15
*191	50	26.2	141	19	9.9	122	1	0.5	36	42	43
354	112	31.6	242	42	11.9	200	3	0.8	67	62	68
274	85	31.0	189	31	11.3	158	2	0.7	57	48	51
476	137	28.8	339	37	7.8	302	8	1.7	103	82	109
1654	497	30.0	1157	177	10.7	980	15	0.9	331	292	342

* When an experiment has been repeated, the results of the second experiment have been tabled with those of the first. The spacing between lines, as shown in this table and similar ones that follow, is meant to separate two such results.

of Doctor STURTEVANT. For experimental matings, males from the forked stock were crossed to Notch females from the separate Notch stocks. Not-Notch $s_c\ b_r\ w^a\ e_c\ r_b$ sisters were mated to forked males and formed the control of the experiments. As will be seen in the data, only two of the four Notches were used in the experiment: Notch 8 represents the type of the longer affected region and Notch 19, the shorter.

TABLE 2

Notch 8 control, $s_c\ b_r\ w^a\ e_c\ r_b$ ♀ × forked ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+♀	+♂	N♀
137	14	10.2	123	15	10.9	108	2	1.6	50	56	..
189	25	13.2	164	12	6.3	152	2	1.1	73	77	..
182	13	7.1	169	9	4.9	160	1	0.5	81	78	..
207	6	2.9	201	5	2.4	196	1	0.5	109	86	..
157	2	1.3	155	5	3.2	150	1	0.6	71	78	..
152	1	0.7	151	22	14.5	129	3	2.0	68	58	..
1024	61	5.9	963	68	6.6	895	10	1.0	452	433	..

TABLE 3

Notch 19/$s_c\ b_r\ w^a\ e_c\ r_b$ ♀ × forked ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+♀	+♂	N♀
104	33	31.8	71	9	8.6	62	1	1.0	21	17	23
128	42	33.8	86	18	14.0	68	1	0.8	22	22	23
28	9	32.0	19	6	21.4	13	1	3.6	5	3	4
133	42	31.6	91	32	24.0	59	1	0.8	29	19	19
83	29	35.0	54	9	10.8	45	1	1.2	17	14	13
87	51	58.5	36	10	11.5	26	1	1.1	9	6	10
128	32	25.0	96	5	3.9	91	0	0.0	29	29	33
172	122	71.0	50	8	4.6	42	0	0.0	8	14	20
863	360	41.7	503	97	11.2	406	6	0.7	131	124	145

TABLE 4

Notch 19 control, $s_c\ b_r\ w^a\ e_c\ r_b$ ♀ × forked ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+♀	+♂	N♀
95	5	5.3	90	9	9.5	81	7	7.4	39	35	..
42	1	2.4	41	7	16.7	34	1	2.4	18	15	..
117	5	4.3	112	9	7.7	103	4	3.4	43	56	..
196	21	10.7	175	14	7.1	161	1	0.5	92	68	..
110	19	17.3	91	18	16.4	73	0	0.0	37	36	..
175	27	15.4	148	42	24.0	106	1	0.6	60	45	..
735	78	10.6	657	99	13.5	558	14	1.9	289	255	..

As shown in the above tables, the percentage of zygotes that died in larval and pupal stages is about the same in the experiments and in their respective controls. We do find, however, a great difference between the experimental and control series in the number of eggs that died, a difference amounting to approximately 25 percent of the total number of eggs. Since no Notch male appeared in the experiments, and since the sex ratio in the experiments was 2 females to 1 male and 1 to 1 in the controls, it is evident that the quarter of the zygotes that died as eggs were males that received the deficient X chromosome from the mother, namely, the Notch males.

Between Notch 8 and Notch 19, there was probably no difference in respect to the fate of the Notch males. A slightly higher general mortality (presumably in both males and females, both Notch and not-Notch) in some of the matings in which Notch 19 was used may be accounted for as due to other modifiers in the stock. This is borne out by the fact that in the control matings of the Notch 19 experiment the same high mortality is present.

The result of the experiments on Notches is summarized in table 5 as follows:

TABLE 5

Summary of the Notch experiments.

CROSSES	EGGS	DISTRIBUTION OF DEATHS				ADULTS			REMARKS
		Eggs	Larvae	Pupae	Total	+♀	+♂	*N* ♀	
N 8♀×f♂	1654	30.0	10.7	0.9	41.6	331	292	342	Notch males
N19♀×f♂	863	41.7	11.2	0.7	53.6	131	124	145	died as eggs
Totals	2517	34.1	10.9	0.8	45.8	462	416	487	
N8 control	1024	5.9	6.6	1.0	13.5	452	433	..	Controls
N19 "	735	10.6	13.5	1.9	26.0	289	255	..	
Totals	1759	7.9	9.5	1.3	18.7	741	688	..	

Autosomal deficiencies

Most of the sectional deficiency cases have been found in the X chromosome and are accompanied by a 2 : 1 sex ratio, for a male that receives a deficient X chromosome does not survive, as was illustrated in the Notch deficiencies. There is nothing in the Y chromosome that counterbalances the deleterious effect of these deficiencies, or, in other words, the Y chromosome contains no gene that replaces the losses incurred in the X chromosome.

Deficiencies present in the autosomes would be easily overlooked, unless the sections lost were so extensive that dominant character changes were produced, or unless such deficiencies were inbred, in which case a lethal effect would be manifest in homozygous condition. Two autosomal deficiencies that give dominant character changes have been found by BRIDGES and are here used to test the fate of the homozygous deficient types.

Plexate

Like Notch, Plexate is a mutation that affects the wings, causing a slight plexus condition of the veins. A single Plexate female was found by BRIDGES (June 16, 1922) in a cross of attached X female and normal male (data unpublished). The Plexate character proved to be dominant in outcrosses and to be lethal when homozygous, that is, when one Plexate is mated to another, the expected homozygous Plexate class is missing, giving only heterozygous Plexates and normal sibs in a 2 : 1 ratio. The viability of the mutant in heterozygous condition is excellent. By linkage tests, the gene for Plexate was found to be situated in the right end of the second chromosome. When BRIDGES crossed Plexate to speck, all the F_1 Plexates were exaggerated and gave pseudo-dominance for speck. The same relation held for balloon. A region from speck to balloon (0.5 ±unit) therefore gives the minimum measure of the deficient section.

In order to compare this case of autosomal deficiency with that of Notch, a test of the homozygous and heterozygous Plexates was made. The Plexate stock was first rearranged by outcrossing Plexate males to sooty females of the inbred sooty stock (See Appendix p. 76) for two successive generations. The F_2 sooty Plexate females were then back-crossed to the sooty stock, in order to substitute by crossing over a part

TABLE 6

Sooty Plexate ♀ × *sooty Plexate* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*Px*	
									♀	♂	♀	♂
302	85	28.1	217	54	17.9	163	1	0.3	25	26	63	48
387	115	29.8	272	56	14.5	216	4	1.0	31	47	78	56
123	35	28.4	88	6	4.9	82	0	0.0	17	14	26	25
198	59	28.9	139	34	17.2	105	3	1.5	19	15	38	30
263	76	28.9	187	37	14.6	150	4	1.5	26	20	46	54
210	55	26.2	155	27	12.9	128	4	1.9	19	26	40	39
258	68	26.4	190	28	10.8	162	2	0.8	21	28	59	52
1741	493	28.3	1248	242	13.9	1006	18	1.1	158	176	350	304

of the second chromosome. Two more backcrosses with the females were thus made before the following analyses were carried out: The experimental series consisted of three types of matings, namely, Plexate by Plexate, Plexate ♀ by sooty ♂ and sooty ♀ by Plexate ♂; the controls were reciprocal crosses between not-Plexate sibs and pure sooty flies.

TABLE 7
Sooty Plexate ♀ × *sooty* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*Px*	
									♀	♂	♀	♂
341	18	5.3	323	45	13.2	278	2	0.6	72	76	69	59
314	38	12.1	276	47	15.0	229	3	0.9	64	57	53	52
237	27	11.4	210	34	14.3	176	0	0.0	47	39	38	52
166	19	11.5	147	25	15.0	122	0	0.0	33	29	33	27
1058	102	9.6	956	151	14.3	805	5	0.5	206	201	193	190

TABLE 8
Sooty ♀ × *Sooty Plexate* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*Px*	
									♀	♂	♀	♂
296	8	2.7	288	22	7.4	266	2	0.7	74	65	69	56
220	5	2.3	215	14	6.4	201	0	0.0	57	45	48	51
288	6	2.1	282	37	12.8	245	2	0.7	56	55	68	64
307	11	3.6	296	33	10.7	263	2	0.7	56	67	69	69
1111	30	2.7	1081	106	9.5	975	6	0.5	243	232	254	240

TABLE 9
Sooty Not-Plexate ♀ × *Sooty* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*Px*	
									♀	♂	♀	♂
302	23	7.6	279	33	10.9	246	1	0.3	123	122	..	..
340	16	4.7	324	39	11.5	285	0	0.0	152	133	..	..
296	7	2.4	289	40	13.5	249	4	1.4	126	119	..	..
938	46	4.9	892	112	11.9	780	5	0.5	401	374	..	..

Between the controls and the sooty ♀ by Plexate ♂ series (tables 8, 9, 10), no significant difference could be found in the number of deaths in any of the three developmental stages. It is evident, therefore, that heterozygous Plexates all came through under the experimental conditions. Comparison of the reciprocal crosses of the experimental series

(compare table 7 with table 8) shows that when Plexate females were used, more of the flies died in both egg (6.9 percent) and larval stages (4.8 percent). This additional mortality was apparently due to the "maternal effect" of the Plexate females.

In the Plexate by Plexate series (table 6), the percentages of mortality occurring in larval and pupal stages were not appreciably different from those in the controls. But in the egg stage, there were approximately 25 percent more of the zygotes that died in the experiment than in the controls. The conclusion seems logical that the homozygous Plexate flies died as eggs.

TABLE 10
Sooty ♀ × *Sooty Not-Plexate* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*Px*	
									♀	♂	♀	♂
301	10	3.3	291	27	9.0	264	0	0.0	133	131	..	..
280	8	2.9	272	23	8.2	249	2	0.7	135	112	..	..
581	18	3.1	563	50	8.6	513	2	0.3	268	243	..	..

When such unhatched eggs were examined under high power, it was found that the embryos had reached a distinctly advanced stage in their development. They showed well developed pharyngeal apparatus and setae on the transverse body ridges. Even the tracheal system was more or less complete. These eggs invariably turned reddish brown and disintegrated. Since the egg mortality in the present case was only slightly complicated by maternal effect, and since practically 90 percent of the unhatched eggs reached this specific and characteristic stage of development, we can hardly avoid attributing this specific lethal effect to the homozygous deficiency.

It will be noted, in passing, that the results shown in the tables of the present experiment are remarkably consistent in all the pairs tested as compared with those of the Notch experiments. This contrast is due to the difference in the time of which these tests were made, that is, the Plexate experiment was carried out at the very last stage of the present work, whereas the Notch work was done more than a year earlier. In the meantime, the technique of handling the material had been improving. Furthermore, in the case of the Notches, two or even three pairs were put into each vial, and not a single vial was discarded from the final counts, although in some vials all the females had died before the fifth day of the experiment.

Minute-l

"Minutes" are mutations characterized by tinier and slenderer bristles, smaller size of body, late hatching and usually poorer viability than the normal flies. Minutes are dominants that are lethal when homozygous. A brief account of these mutants has been published by MORGAN, BRIDGES and STURTEVANT in the Year Books of the CARNEGIE INSTITUTION from 1923 to 1925. Minute-k, Minute-n and Minute-o were shown to be sex-linked, dominant in the female and lethal in the male and are possibly deficiencies similar to the Notches. Among the autosomal Minutes may be mentioned Minute-h, Minute-i, and Minute-j, located in the third chromosome, Minute-l, Minute-m and Minute-p in the second chromosome. The supposition that many Minutes are probably deficiencies is strengthened by the demonstrative work with Minute-l.

Among the offspring of a cross between a triploid female and a diploid male, BRIDGES found one Minute female (July 7, 1923, result unpublished). He named the mutant Minute-l. Like all other Minutes, it is a dominant character and lethal when homozygous. It gave a reduction of crossing over values in the right end of the second chromosome. Its locus was found to be in the neighborhood of plexus. When it was crossed to plexus, it gave exaggerated Minute-l and plexus compounds. It also showed pseudo-dominance with arc, plexus and arc being about one unit apart. No crossing over has so far been observed between arc and plexus in the presence of Minute-l. Thus, this particular case fulfills the expectations for a typical sectional deficiency.

TABLE 11

Sooty Minute-l ♀ × *sooty Minute-l* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*M-l*	
									♀	♂	♀	♂
205	71	34.6	134	17	8.3	117	8	3.9	26	19	33	31
262	82	31.3	180	44	17.5	130	0	0.0	27	22	37	50
337	115	34.1	222	63	18.7	159	9	2.2	25	30	54	41
122	42	34.4	80	20	16.4	60	1	0.8	10	9	12	28
167	51	30.6	116	21	12.6	95	3	1.8	23	12	31	26
139	48	34.5	91	22	15.8	69	4	2.9	9	12	17	27
177	64	36.2	113	11	6.2	102	4	2.3	21	15	29	33
1409	473	33.6	936	198	14.1	738	29	2.1	141	119	213	236

The analysis of Minute-l, as to the fate of homozygous and heterozygous Minutes, was made simultaneously with the Plexate case. Precisely the same procedure was followed in both cases. The sooty Minutes were

finally used in the tests appearing in tables 11 to 15, and the crosses between not-Minute sibs with the pure sooty formed the controls.

TABLE 12

Sooty Minute-l ♀ × *sooty* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*M-l*	
									♀	♂	♀	♂
243	29	11.9	214	38	15.6	176	7	2.8	40	50	36	43
212	37	17.4	175	42	19.8	133	4	1.9	32	28	35	34
230	34	14.8	196	45	19.6	151	1	0.4	36	35	33	46
130	15	11.5	115	15	11.5	100	2	1.5	35	23	18	22
1029	145	14.1	884	173	16.8	711	16	1.5	175	171	164	185

TABLE 13

Sooty ♀ × *sooty Minute-l* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*M-l*	
									♀	♂	♀	♂
264	7	2.7	257	9	3.4	248	4	1.5	68	63	66	47
311	8	2.6	303	22	7.1	281	5	1.6	66	70	82	58
245	5	2.0	240	16	6.5	224	4	1.6	57	49	54	60
280	13	4.6	267	32	11.4	235	7	2.5	61	61	51	55
1100	33	3.0	1067	79	7.2	988	20	1.8	252	243	253	220

TABLE 14

Sooty not-M-l ♀ × *sooty* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*M-l*	
									♀	♂	♀	♂
280	11	3.9	269	12	4.3	257	4	1.4	161	122	..	..
351	7	2.0	344	38	10.8	306	3	0.9	149	154	..	..
236	5	2.1	231	8	3.4	223	0	0.0	125	98	..	..
867	23	2.7	844	58	6.7	786	7	0.8	405	374	..	..

TABLE 15

Sooty ♀ × *sooty not-M-l* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		*M-l*	
									♀	♂	♀	♂
171	3	1.8	168	4	2.3	164	0	0.0	84	80	..	..
273	7	2.6	266	26	9.5	240	3	1.1	111	120	..	..
444	10	2.3	434	30	6.8	404	3	0.7	195	206	..	..

The results in tables 13, 14 and 15 show that the percentage of mortality in all three developmental stages was practically the same in the sooty ♀ by Minute-l ♂ series as in the controls. The heterozygous Minutes appear, therefore, to have practically all survived under the experimental conditions. A small number (about 1 percent) of the heterozygous Minutes died in an advanced pupal stage, and these when examined were found to be more often females than males. Under the ordinary breeding conditions, Minute-l is generally only about 60–70 percent as viable as normals. The improvement of the viability of the Minute-l flies in the present experiment may be attributable to a greater proportional amount of food, less larval crowding and to other more favorable environmental conditions of the vial method as contrasted to the usual milk bottle method of culture. Heterozygous Minutes, however, emerged later than their normal sibs.

When the Minute-l by Minute-l series is compared with the controls (tables 11, 12, 13 and 14) no significant difference in percentages of deaths in either the larval or the pupal stage is found. There was, however, an excess of approximately 25 percent of the zygotes that died in the egg stage in the experiment as compared with the deaths of eggs in the controls. The quarter of the total number of flies that died as eggs were presumably homozygous Minutes.

In all cases where the Minute-l females were used in the crosses (tables 11 and 12), there was a remarkably higher percentage of egg and larva mortality than in cases where the sooty females were used. This is an example of maternal effect. The Minute-l females regularly lay abnormal eggs, comparable to those observed in singed females (MOHR 1922). The eggs vary in size and shape through a wide range, but they do not approach those of the normal at one extreme, nor are they quite like the eggs of singed at the other. They are, as a rule, smaller than normal in size and more oval in shape, with a lesser degree of concavity on the dorsal side. The "floats" are more or less normal, but sometimes appear thinner and more delicate. By far the most interesting feature of these eggs is the character of the yolk. Instead of being opaque white as in normal eggs, the yolk is nearly transparent with finer glassy granules. The developing embryo inside of the egg is often visible. Most of the eggs on the extreme abnormal side failed to hatch or else hatched as undersized larvae. The percentages of such extremely abnormal eggs and of mortality resulting from maternal effect were very close.

Sectional duplications

The first "duplication" case in Drosophila was reported by BRIDGES (1919). It was interpreted as having arisen by a section of the X chromosome, including the loci for vermilion and sable, becoming detached from its normal location in the middle of the chromosome and jointed on to the "zero" end of its mate. This interpretation is challenged by the recent work of BONNIER (1926), who suggests that this so-called duplication is due to gene mutation, that is, to a recessive suppressor similar to others known (tan suppressor), rather than to a dislocation of allelomorphic genes. On the other hand, Pale translocation seems to be well established as a clear-cut case involving duplication.

Pale translocation

In preparing some stocks of sex-linked characters for work with lethal tumors, BRIDGES found that in one culture there were flies present with a light yellow eye color in addition to the expected eosin flies. These "Pale" flies were subsequently shown to be a specific dilution of eosin. Later, through extensive crosses, BRIDGES was led to the conclusion that the peculiar phenomenon encountered could be explained as due to the transference of a piece from the right end of the second chromosome to an attachment near the right end of the third chromosome, a rare case of autosomal translocation (MORGAN and BRIDGES 1925).

Through study of the dominance relations of genes, it has been shown that for most mutant characters one dominant gene is the suppressor of two recessives. Hence the wild-type genes in the duplicating section act as dominant suppressors of all the mutants that lie to the right of arc in the second chromosome, including plexus, lethal-IIa, brown, blistered, purploid, morula, speck and balloon, amounting to seven units on the map. This is an accurate measure of the duplicating fragment. A further proof is found in the fact that a second chromosome which has lost its right end cannot undergo crossing over in that region with the normal homologous chromosome.

A cytological analysis seemed to show that the shorter pair of V's has one arm abnormally short (the right end of the second chromosome). It has not yet been established, however, that the third chromosome is increased by a corresponding section, (MORGAN, BRIDGES and STURTEVANT 1925).

This stock was examined by the author in the latter part of 1924. At first two recessive characters, plexus and speck, were used as an index of the different kinds of combinations of the sectional deficiencies and

sectional duplications. Unfortunately, the plexus speck chromosome was found to contain a certain recessive modifier that killed off a very high proportion of the zygotes at an early stage. About 75 percent of the eggs failed to hatch in the control series consisting of plexus speck by plexus speck matings. After many fruitless attempts to eliminate this modifier, the stock was finally abandoned; instead of using plexus and speck as an index, only plexus, of different origin, was used in the following work.

The locus of plexus is in that part of the second chromosome that has been transferred to the third chromosome. In the diagram of the relations in the Pale stock (figure 1) the section of the second chromosome (broad line) joined to the third chromosome (thin line) near its end carried a wild-type dominant allelomorph of plexus. Each of the normal second chromosomes of this stock (full length broad line) carries a recessive plexus gene (locus indicated by an arrow head). The second chromosome from which the end has been removed is indicated by the shorter broad line with the dotted end.

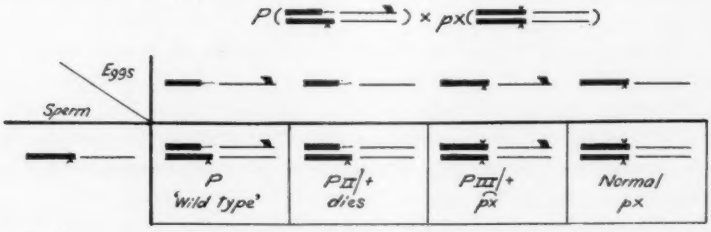

FIGURE 1.

As seen in figure 1, there are three classes of adults in the Pale stock, namely, (1) the Pale translocation (symbol: *P*), (2) the heterozygous duplication (symbol: $P_{III}/+$) and (3) the normal. In the absence of speck and eosin, the task of differentiating these classes depends on the accurate separation of (1) the heterozygous plexus representing the Pale from (2) the semi-plexus (symbol: s-*px*) representing the heterozygous duplication, in which the homozygous plexus is partly suppressed by an allelomorphic wild-type gene, and (2) semi-plexus from (3) the homozygous plexus representing the normal condition of the chromosomes. While the heterozygous plexus is phenotypically a wild type and easily distinguished, the separation of semi-plexus from homozygous plexus is by no means easy, as there is a considerable variation in the expression of the plexus character in homozygous condition.

A careful comparative study of a homozygous plexus stock with that in which 40–50 percent of the flies were expected to be of the semi-plexus type led to the discovery of a reliable criterion. In the first place, the degree of the plexus condition in the semi-plexus type is generally considerably less than in the homozygous type, but an infallible character is found in the differences in the second posterior cell of the wing. In the homozygous plexus, no matter how near it may approach otherwise in appearance to the semi-plexus condition, the posterior crossvein invariably branches out into the posterior cell, whereas in semi-plexus the cell is clean like that of the normal wings. This was made certain by testing many of these supposed semi-plexus individuals, and in every case their offspring gave the expected results.

With the Pale, the semi-plexus and homozygous plexus types differentiated and the stock purified by an adequate amount of inbreeding and selection, experiments were planned to test the fate of the different aberrations. This was done by mating these three classes of flies in all possible combinations. Six series of crosses were thus made, three of which were with reciprocal crosses:

1. Homozygous plexus by homozygous plexus of course forms the control of the experiments, all of the flies in this series have normal chromosomes and are expected to reach the adult stage. The proportions of deaths of the homozygous plexus class that occur in this control may therefore be expected to occur in each of the other experiments in addition to the deaths due to the specific chromosome aberrations.

2. A cross of homozygous plexus with semi-plexus gives in one-half of their progeny heterozygous duplication on the third chromosome ($P_{III}/+$) which, according to BRIDGES, is only 80 percent as viable as the normal under ordinary culture conditions.

3. When semi-plexus is mated to semi-plexus, one-quarter of the offspring receives both of the duplication-bearing third chromosomes (symbol: P_{III}/P_{III}). These flies die. The ratio of adults expected from such a cross, therefore, is approximately two semi-plexus to one homozygous plexus.

4. In the homozygous plexus by Pale cross (see figure 1), one-quarter of the progeny contains both the deficient second chromosome and the duplication-bearing third chromosome and reconstitutes the Pale class. Another quarter receives the deficient second chromosome. The heterozygous condition for Pale deficiency (symbol: $P_{II}/+$) is lethal. A third quarter receives the duplication-bearing third chromosome and forms the semi-plexus class. Finally, the fourth quarter, having both the normal

second and the normal third chromosomes, gives the homozygous plexus class with normal chromosomes and viability.

5. A more complicated situation is to be found in the cross between semi-plexus and Pale. The number and kind of zygotes produced by such a cross are shown in figure 2.

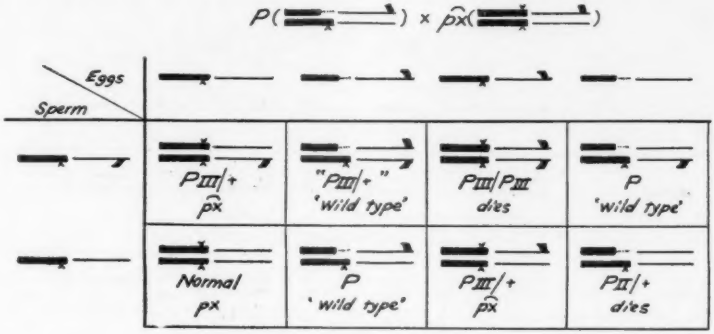

FIGURE 2.

As seen in the above diagram, two of the eight combinations are fatal, namely, homozygous duplication of the third chromosome (P_{III}/P_{III}), and the heterozygous deficiency of the second chromosome ($P_{II}/+$), amounting together to 25 percent of the total number of zygotes. The remaining three-quarters, that survive, consist of three classes, the heterozygous plexus, semi-plexus and homozygous plexus in a zygotic ratio of 3 : 2 : 1. Of the heterozygous plexus class, two-thirds are Pales of the usual type and one-third is of the constitution in which a homozygous duplication is partly balanced by a heterozygous deficiency (symbol "$P_{III}/+$"). This last type of heterozygous plexus is, consequently, expected to be only 80 percent as viable as the normal under ordinary conditions.

6. Finally, when two Pale flies are mated together, there will be sixteen possible combinations of gametes, as illustrated in figure 3.

Seven of the sixteen types of zygotes die, including the heterozygous deficiency ($P_{II}/+$), the homozygous deficiency partly balanced by heterozygous duplication (symbol: "$P_{II}/+$"), homozygous deficiency (P_{II}/P_{II}), homozygous deficiency with homozygous duplication (symbol: P_{II} P_{III}/P_{II} P_{III}) and homozygous duplication (P_{III}/P_{III}), the first two types named having two classes each. The total mortality, therefore,

amounts to about 48 percent. Among the nine classes of flies that reach the adult stage, there are six heterozygous plexus, two semi-plexus and one homozygous plexus. Of the heterozygous plexus again, two-thirds are Pales and one-third heterozygous duplications ("$P_{III}/+$").

The results of these six series of matings (three having reciprocal crosses) are shown in tables 16 to 24 inclusive.

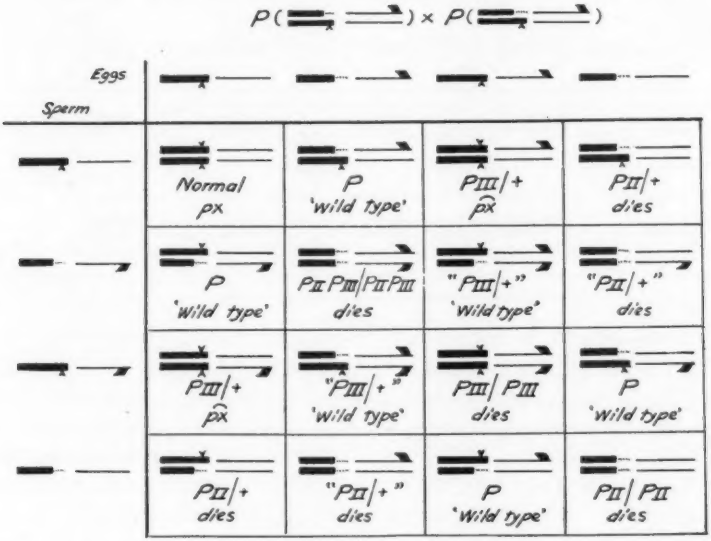

FIGURE 3.

As seen in the following tables, a considerable number of individuals died as molts. The word "molt" is used here to designate flies that have undergone the last larval molt but have not yet succeeded in forming definite puparia. At the time when the adults began emerging, a few larvae, presumably hatched from the same batch of eggs, were seen still crawling around before attempting to form puparia. Presently such larvae came to rest and molted. These belated individuals never formed definite puparia and died in this molt stage. The interior larval tissue usually disintegrated, but sometimes it was found shriveled and dried up, sticking to one side or to the bottom of the molt-case. At times, such a dried mass, after dissection from the molt-case, showed a large brown spot, suggesting that the imaginal discs had started development.

TABLE 16

Plexus ♀ × plexus ♂ (control).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	pz♀	pz♂
163	3	1.8	164	4	2.4	160	11	6.6	149	2	1.3	59	88
233	3	1.3	230	13	5.6	217	12	5.1	205	0	0.0	97	108
214	10	4.7	204	2	1.0	202	3	1.4	199	1	0.5	99	99
213	5	2.3	208	7	3.3	201	1	0.5	200	0	0.0	108	92
238	10	7.8	228	4	1.7	224	1	0.4	223	0	0.0	111	112
128	4	3.1	124	6	4.7	118	1	0.8	117	0	0.0	61	56
148	2	1.3	146	1	0.7	145	1	0.7	144	0	0.0	65	79
1341	37	2.8	1304	37	2.8	1267	30	2.2	1237	3	0.3	600	634

TABLE 17

Plexus ♀ × semi-plexus ♂ (second series).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*s-pz*		*pz*	
												♀	♂	♀	♂
496	8	2.7	288	25	8.4	263	5	1.7	258	4	1.3	60	58	65	71
302	4	1.3	298	22	7.3	276	8	2.6	268	1	0.3	69	61	81	56
268	2	0.7	266	8	3.0	258	1	0.4	257	2	0.8	59	60	83	53
145	1	0.7	144	4	2.8	140	4	2.8	136	0	0.0	28	45	35	28
205	4	2.0	201	5	2.5	196	3	1.5	193	0	0.0	40	53	55	45
200	12	6.0	188	7	3.5	181	2	1.0	179	1	0.5	49	40	47	42
233	1	0.4	232	14	6.0	218	2	0.9	216	1	0.4	55	57	53	50
1649	32	1.9	1617	85	5.2	1532	25	1.5	1507	9	0.5	360	374	419	345

TABLE 18

Semi-plexus ♀ × plexus ♂ (reciprocal).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*s-px*		*px*	
												♀	♂	♀	♂
245	34	13.9	211	31	12.6	180	4	1.6	176	5	2.0	40	40	54	37
271	23	8.5	248	43	15.8	205	2	0.7	203	5	1.8	51	45	56	46
187	3	1.6	184	21	11.2	163	3	1.6	1609	7	3.7	26	45	51	31
703	60	8.5	643	95	13.5	548	9	1.3	53	17	2.4	117	130	161	114

TABLE 19

Semi-plexus ♀ × semi-plexus ♂ (third series).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*s-px*		*px*	
												♀	♂	♀	♂
242	24	9.9	218	75	34.4	143	6	2.5	137	5	2.1	40	36	35	21
217	33	15.2	184	55	25.3	129	4	1.8	125	3	1.4	26	41	35	20
194	17	8.8	177	54	31.0	123	4	2.1	119	4	2.1	21	38	31	25
653	74	11.3	579	184	28.4	395	14	2.1	381	12	1.8	87	115	101	66

TABLE 20

Plexus ♀ × Pale ♂ (fourth series).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*P*		*s-px*		*px*	
												♀	♂	♀	♂	♀	♂
267	8	3.0	259	54	20.2	205	11	4.1	194	3	1.1	30	36	28	27	35	35
191	5	2.6	186	41	21.5	145	19	9.9	126	0	0.0	26	25	16	13	23	23
273	5	1.8	268	46	16.9	222	16	5.9	206	1	0.4	39	37	31	28	35	35
150	5	3.3	145	40	26.7	105	16	10.7	89	0	0.0	13	15	13	18	15	15
181	5	2.8	176	43	23.8	133	12	6.6	121	0	0.0	19	21	23	17	21	20
153	2	1.3	151	26	17.0	125	15	9.8	110	0	0.0	18	22	13	19	15	23
1215	30	2.5	1185	250	20.6	935	89	7.3	846	4	0.3	145	156	124	122	144	151

1215	30	2.5	1185	250	20.6	935	89	7.3	846	4	0.3	145	156	124	122	144	151

TABLE 21

Pale ♀ × plexus ♂ (reciprocal).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*P*		*s-pz*		*pz*	
												♀	♂	♀	♂	♀	♂
322	1	0.3	321	47	14.6	274	33	10.2	241	2	0.6	39	43	25	47	50	35
330	5	1.5	325	62	18.8	263	32	9.7	231	2	0.6	47	33	36	36	41	36
272	8	2.9	264	37	13.6	227	29	10.7	198	0	0.0	39	29	30	26	40	34
272	4	1.4	268	50	18.4	218	26	9.6	192	0	0.0	32	36	23	37	29	35
289	4	1.4	285	56	19.4	229	34	11.8	195	0	0.0	24	49	31	30	39	22
311	2	0.6	309	50	16.1	259	28	9.0	231	3	1.0	52	36	21	44	44	31
218	5	2.3	213	32	14.7	181	25	11.5	156	0	0.0	23	21	29	32	24	27
2014	29	1.4	1985	334	16.6	1651	207	10.3	1444	7	0.3	256	247	195	252	267	220

TABLE 22

Pale ♀ × semi-plexus ♂ (fifth series).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*P*		*s-pz*		*pz*	
												♀	♂	♀	♂	♀	♂
396	3	0.8	393	102	25.8	291	17	4.3	274	10	2.5	91	56	41	27	25	24
198	5	2.5	193	41	21.7	152	15	7.6	137	7	3.5	37	27	25	19	15	7
298	2	0.7	296	63	21.1	233	37	12.4	196	0	0.0	43	38	37	31	28	19
243	3	1.2	240	58	23.5	182	19	7.8	163	3	1.2	42	46	27	18	14	13
216	3	1.4	213	49	22.7	164	29	13.4	135	0	0.0	29	38	30	27	10	11
1351	16	1.2	1335	313	23.1	1022	117	8.7	905	20	1.5	242	205	150	122	92	74

TABLE 23

Semi-plexus ♀ × Pale ♂ (reciprocal).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*P*		*s-px*		*px*	
												♀	♂	♀	♂	♀	♂
166	8	4.8	158	36	21.7	122	9	5.4	113	1	0.6	34	30	18	18	6	6
264	15	5.7	249	63	23.9	186	23	8.7	163	1	0.4	32	57	30	18	15	10
193	16	8.3	177	47	24.4	130	7	3.6	123	1	0.5	37	29	15	13	11	17
175	15	8.6	160	44	25.0	116	6	3.4	110	2	1.1	19	28	24	17	10	10
121	2	1.7	119	33	27.2	86	10	8.3	76	1	0.8	23	13	16	10	9	4
250	10	4.0	240	72	28.8	168	9	3.6	159	1	0.4	40	38	28	20	19	13
1169	66	5.6	1103	295	25.2	808	64	5.5	744	7	0.6	185	195	131	96	70	60

TABLE 24

Pale ♀ × Pale ♂ (sixth series).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	MOLTS	MOLTS DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	*P*		*s-px*		*px*	
												♀	♂	♀	♂	♀	♂
276	35	12.7	241	86	31.2	155	16	5.8	139	7	2.5	42	49	15	11	10	5
242	28	11.6	214	76	31.4	138	13	5.4	125	10	4.1	38	40	16	12	3	6
228	21	9.3	207	73	32.2	134	11	4.9	123	6	2.7	33	42	15	19	6	2
210	18	8.6	192	61	29.0	131	16	7.6	115	8	3.8	38	32	10	14	7	6
217	23	10.6	194	64	29.4	130	12	5.5	118	12	5.5	33	35	10	15	8	5
226	23	10.2	203	48	21.2	155	16	7.1	139	5	2.2	37	41	19	17	8	12
184	22	11.9	162	45	24.4	117	15	8.2	102	11	6.0	30	34	7	11	3	6
244	26	10.7	218	63	25.8	155	19	7.8	136	10	4.1	44	39	9	17	8	9
288	22	7.6	266	81	28.1	185	21	7.3	163	8	2.8	55	54	17	19	7	4
229	29	12.7	200	50	21.8	150	24	10.5	126	5	2.2	34	43	17	14	7	6
2344	247	10.5	2097	647	27.6	1450	163	6.9	1287	82	3.5	384	409	135	149	67	61

The results shown in the foregoing tables are summarized in table 25.

TABLE 25

Summaries of pale translocation.

SERIES	CROSSES	EGGS	DISTRIBUTION OF DEATHS					ADULTS		
			Eggs	Larvae	Molts	Pupae	Total	*P*	*s-px*	*px*
1	px ♀ × px ♂	1341	2.8	2.8	2.2	0.3	8.1	...	...	1234
2	px ♀ × s-px ♂	1649	1.9	5.2	1.5	0.5	9.1	...	734	764
	s-px ♀ × px ♂	703	8.5	13.5	1.3	2.4	25.7	...	247	275
3	s-px ♀ × s-px ♂	653	11.3	28.4	2.1	1.8	43.6	...	202	167
4	px ♀ × P ♂	1215	2.5	20.6	7.3	0.3	30.7	301	246	295
	P ♀ × px ♂	2014	1.4	16.6	10.3	0.3	28.6	503	447	487
5	P ♀ × s-px ♂	1351	1.2	23.1	8.7	1.5	34.5	447	272	166
	s-px ♀ × P ♂	1169	5.6	25.2	5.5	0.6	36.9	380	227	130
6	P ♀ × P ♂	2344	10.5	27.6	6.9	3.5	48.5	793	284	128

From the results of table 25, the following conclusions seem evident:

1. In the control series of plexus by plexus (1), a total of about eight percent of the eggs died before attaining the adult stage. These deaths were distributed in all stages evenly, except that, as is usual, very few deaths occurred in the pupal stage.

2. In the homozygous plexus ♀ by semi-plexus ♂ series (2, upper line), the percentage of deaths in all developmental stages was only slightly greater than in the control (1), and the ratio of the semi-plexus to homozygous plexus was slightly below one to one. It follows that under the experimental conditions about 95 percent of the heterozygous duplication flies ($P_{III}/+$) lived. Since these flies are only 80 percent viable under ordinary culture conditions, the increase of viability here must be taken to mean that the better environmental conditions of the vial-culture method are responsible for the full proportion of flies reaching adult stage. The semi-plexus flies invariably began to emerge about a day later than the other flies.

3. Comparing the reciprocal cross of semi-plexus ♀ by homozygous plexus ♂ (2, lower line), we find there has been a general increase of mortality in the egg, larval and pupal stages in cases where the semi-plexus females were used. This may be interpreted as a maternal effect of the heterozygous duplication females. This effect was impressed upon all eggs, since the deaths apparently occurred nearly at random in both semi-plexus and homozygous plexus classes. It is probable that the maternal effect shown in this particular experiment was greater than was the case where semi-plexus females were used in the other crosses.

4. In the semi-plexus by semi-plexus series (3), there were 8.5 percent zygotes more that died as eggs and 25.6 percent more that died as larvae than died in the control (1). About half of the deaths occurring in the egg stage can safely be attributable to the maternal effect of the semi-plexus females. Likewise about a quarter of the zygotes that died as larvae may be attributed to maternal effect. In this case the maternal effect apparently bore more heavily upon the semi-plexus than on the homozygous plexus zygotes, for while the ratio of the *s-px* to *px* was expected to be 2 : 1, it actually was only 1.2 : 1. This was the lowest survival ratio for semi-plexus that was observed in the eight crosses in which the type occurred. The excess of deaths, namely, about 5 percent in the egg stage and 20 percent in the larval stage, is to be attributed to the homozygous duplication flies (P_{III}/P_{III}), which should constitute 25 percent of the zygotes and which are known to die.

5. Between the homozygous plexus ♀ by Pale ♂ series (4, upper line), no appreciable difference was shown in percentage of deaths in either egg or pupal stage. But in the larval and molt stages, there was a total of somewhat more than 25 percent of zygotes that died in the experiment than that died in the control. The *s-px* type was expected to constitute a third of the zygotes, and it fell short of its quota by only 35 flies or 3 percent of the total eggs. It is therefore certain that the bulk of the mortality in larval and molt stages was due to deaths of the heterozygous Pale deficiency ($P_{II}/+$). Especially characteristic of the heterozygous P_{II} zygotes was the occurrence of death in the molt stage.

6. A careful examination of the reciprocal cross of Pale ♀ by homozygous plexus ♂ series (4, lower line) brings out the fact that when Pale females were used relatively more flies died as molts and relatively fewer as larvae. This deference of the time of death seems to have occurred consistently in all classes (table 21), and may be taken to mean that the Pale had less maternal effect than even the homozygous plexus. Correlated with the slighter maternal effect in this reciprocal cross was a higher viability of the semi-plexus class which failed to fill its quota by only 32 flies or 1.5 percent of the number of eggs.

7. Comparing the Pale ♀ by semi-plexus ♂ series (5, upper line) with the control, we find in the former no more deaths in the egg stage. There was, however, about 20 percent more deaths in the larval and about 5 percent more deaths in the molt stage. From the analysis given in figure 2, it is seen that 12.5 percent of the zygotes should die from homozygosis for P_{III} and another 12.5 percent should die from heterozygosis for P_{II}. Accordingly, the 25 percent of excess deaths observed in this experi-

ment are no more than can be attributed to the heterozygous Pale deficiency ($P_{II}/+$) and the homozygous duplication (P_{III}/P_{III}) zygotes. Moreover, the ratio among the surviving adults approximated the 3 *P* : 2 *s-px* : 1 *px* ratio expected. From this it is clear that the mortality of the "$P_{II}+/P_{III}\ P_{III}$" form is slight, probably little greater than that of the $P_{III}/+$type. This was to be expected since an added P_{III} (a duplication) is neutralized by an added P_{II} (the corresponding deficiency).

8. The results of the reciprocal cross, *s-px* ♀ by *P* ♂ (5, lower line), support the above conclusions, and also show that whenever semi-plexus females were used the total death rate was increased by 2 percent, and that the distribution of death was shifted more toward the earlier stages. The ratio of the Pale, semi-plexus and homozygous plexus classes was approximately 3 : 2 : 1, thus agreeing with the expected results.

9. Finally, in the Pale by Pale series, there were 10.5 percent of zygotes that died as eggs, 27.6 percent as larvae, 6.9 percent as molts and 3.5 percent as pupae, totalling 48.5 percent, which agrees almost exactly with the calculated value (48 percent, see figure 3). The fact that the results here were even better than expected from the control series is not particularly surprising, when we recall that, in series 4 and its reciprocal, Pale females were better mothers than the homozygous plexus. An especially good evidence of the relatively low general mortality is the fact that the semi-plexus type more than filled its quota.

As seen in figure 3, when Pales are mated to each other, there are five classes in the offspring that are expected to die. Two of the five, namely, homozygous deficiency (P_{II}/P_{II}) and homozygous deficiency with homozygous duplication ($P_{II}\ P_{III}/P_{II}\ P_{III}$) total 12.5 percent (that is, 6.25 percent each). Assuming that the homozygous P_{II} has a more deleterious effect than the others, we may conclude that zygotes receiving both deficient second chromosomes probably all died as eggs. The remaining 4 percent of mortality in the egg stage must be mainly due to the presence of flies that were homozygous for the deficient second chromosomes and at the same time for the duplication bearing third chromosomes. From the percentage that died as eggs and the large percentage that died as larvae, it appears that about one-third of these zygotes had hatched into larvae and died. The fact that these flies die supports BRIDGES' interpretation that the duplicating section is not quite as great as the deficiency, and that $P_{II}\ P_{III}/P_{II}\ P_{III}$ is, in net effect, a homozygous deficiency.

Summary of the sectional aberrations

Before passing to the next part of the present work, in which a slightly different category of the chromosome aberrations is dealt with, it may be well to restate the more important conclusions thus far reached:

1. The analysis of the Pale translocation showed several cases (heterozygous P_{II}, homozygous P_{III} and homozygous P_{II} with heterozygous P_{III}) in which the deaths occurred late in development at the larval or even molt stages, and at a rather definite stage in each case. An analogous case is found in the fate of the hybrid males in the species cross between *D. melanogaster* and *D. simulans*. According to STURTEVANT (1921), the male hybrid larva grows slowly, rarely pupates and then forms only an abnormal puparium (probably in the "molt" stage of the present work). A histological analysis of the hybrid larvae has led BONNIER (1924) to conclude that "a suspension of the normal synchrony between the development of the different organs" may be directly responsible for the death of the larvae. His account is based on only three pathological specimens; and as STURTEVANT pointed out, the evidence is too meager to carry weight.

2. It has been demonstrated in the various preceding sections that heterozygous deficiencies of lesser extent, such as Notches, Plexate and Minute-l, all had little effect upon the success of development, aside from the fact that the deficiency bearing females showed varying degrees of irregularity in their reproductive activity, including a general mortality of eggs and larvae of all classes at random, which is designated by the term "maternal effect."

3. Among the four cases analyzed, the Notches (0.1–3.0 units) Plexate (0.5 unit) and Minute-l (1.0 unit) may be said to be minor deficiencies, in which the deficiency in one chromosome is nearly balanced by the normal condition of the allelomorphic genes of the homologous chromosome. The Minute-l case (1.0 unit) may be considered as on the borderland. Under very favorable conditions probably all the heterozygous Minutes lived, but these emerged later than their normal sibs. In the Pale deficiency case, the section involved is seven units in length, that is, several times as long as that of the minor deficiencies. The consequence is that none of the heterozygous Pale deficiency survived and few if any reached the pupal stage. Evidently, there is a graded difference in the lethal effects of the deficiencies; and these differences can be best accounted for in terms of the number and kind of genes lost.

4. The homozygous deficiencies in the autosomes (*Px/Px*, *Ml/Ml* and P_{II}/P_{II}) and the X-chromosome deficiency in the male (Notch males)

have been shown, on the other hand, to be lethal early in the development of the flies. These all showed their deleterious effect in the egg stage. The eggs that failed to hatch were examined under high power in an attempt to determine the specific stage to which the embryos of the different types of homozygous deficiencies had developed. Since varying percentage of the egg mortality was caused by the maternal effect of the deficiency-bearing females, and since the deaths as a result of such effect usually extended from early embryos to larvae immediately before hatching, it was not practical, in most cases, to separate the unhatched eggs due to the specific effect of homozygous deficiencies from those due to the maternal effect. However, in the Plexate case, where the maternal effect was comparatively slight, the evidence clearly showed that the embryos carrying the homozygous deficiency died in a highly specific stage of development.

5. In regard to sectional duplication, as the results in the Pale translocation showed, the heterozygous duplication ($P_{III}/+$) probably all lived under the favorable experimental conditions, although, as a rule, these flies emerged slightly later than their normal sibs and the female showed a higher general mortality in the egg and larvae of their offspring. The homozygous condition of the same duplication, on the other hand, has been shown to be lethal in the larval stage. A comparison of the Pale deficiency and the Pale duplication brings out the fact that the deleterious effect of the deficiency is greater and becomes manifest earlier in the course of development than in the case of the corresponding duplication. This is in accord with the assumption that in duplication cases, the alteration involves mainly quantitative differences in the genes of less proportional extent.

Aberrations that involve a whole chromosome

Through the process of a non-disjunction, duplications or deficiencies of whole chromosomes may arise. Theoretically, all chromosomes are subject to this irregularity in distribution. Since non-disjunction was discovered by BRIDGES (1913) in the X chromosome, close watch has been kept for phenomena that would indicate non-disjunction of the autosomes. Despite the painstaking search, no evidence has so far been found showing non-disjunction of the second or of the third chromosomes. It is probable that cases of second- and third-chromosome non-disjunctions have occurred, but that such aberrations were invariably lethal. The work of the sectional deficiency of Pale translocation seems to support this view; the evidence there shows that the loss of a section from the right

end of the second chromosome is enough to kill the individual in the larval stage. The small fourth chromosome, however, has been found to be subject to non-disjunction.

Deficiency and duplication of the X chromosome

It seems probable that primary non-disjunction of the X chromosome is initiated by some unusual local difference, in consequence of which the synapsed X's lag on the reduction spindle and fail to separate from each other. Owing to the delay in separation both X's may be retained in the egg or else both pass into the polocyte. It is a matter of chance whether the nucleus that receives both X's later becomes the egg nucleus or the nucleus of the polocyte. In the former case, the egg will have two X's; in the latter case, the egg will lack both X chromosomes. When the XX-eggs are fertilized, the chances are equal that an XXX or an XXY zygote results. The O-eggs produce equal numbers of XO and YO zygotes. The XXX individuals are superfemales with a mortality that is usually about 99 percent. The XO individuals are sterile males. The YO zygotes fail to appear at all. The XXY individual is a female that is normal in sex type, but that gives rise to further exceptions to sex-linked inheritance, which process is called secondary non-disjunction. An XXY female produces four kinds of eggs, XX, XY, X and Y. When these are fertilized by sperm from a normal male, eight classes of zygotes are produced. If the X chromosomes of the XXY female carry a recessive gene, say white eyes, and the X of the father carries the dominant wild-type allelomorph, red eyes, then the classes are as follows:

sperm \ eggs	X^wX^w	X^wY	X^w	Y
X^+	$X^wX^wX^+$ red super-♀ usually dies	X^wX^+Y regular red ♀	X^wX^+ regular red ♀	X^+Y exceptional red♂
Y	X^wX^wY exceptional white ♀	X^wYY regular white ♂	X^wY regular white ♂	YY dies

The only way to analyze the effect of these different aberrations is through a mating of the types shown above. The YY-type (nullo-X), representing the deficiency of the X chromosome, invariably dies but the XXX females, representing the duplication of X chromosome, occasionally come through. All the other types are known to be normal in development. The presence of an extra Y does not in any way interfere with the

normal development of the female. Since we are concerned only with the fate of the YY and XXX individuals, the use of the stock in which the X's are permanently attached greatly simplifies the work.

Double-yellow

A strain of yellow flies that has its X chromosomes permanently attached to each other was found by L. V. MORGAN (1922). A cross of a "double-yellow" female gives four classes of zygotes, namely, XXY, XY, XXX and YY. The yellow females of the constitution XXY and the wild-type males XY are expected to come through normally. It should be comparatively easy matter to discover at what stage the YY and the XXX individuals die.

In preparing an experimental stock, double-yellow females were crossed to sooty males for three successive generations, at the end of which time the stock was presumably highly similar to the sooty stock in respect to its autosomal genes. The experiment was then carried out by mating double-yellow sooty females to pure sooty males. Four pairs of sooty brother-and-sister matings constituted the control series.

TABLE 26

Double-yellow sooty ♀ × *sooty* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	YY ♀	♂ ♂
381	105	27.6	276	74	19.4	202	25	6.7	82	95
328	89	27.1	239	57	17.4	182	32	9.8	76	74
340	91	26.8	249	82	24.1	167	16	4.7	74	77
243	66	28.2	177	65	27.8	112	9	3.8	43	60
330	81	24.4	249	75	22.6	174	20	6.0	75	79
296	78	26.4	218	58	19.6	160	15	5.3	61	84
1918	510	26.6	1408	411	21.4	997	117	6.1	411	461

TABLE 27

Sooty ♀ × *sooty* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	♂ ♀	♂ ♂
231	7	3.3	224	31	13.0	193	2	0.9	104	87
236	11	4.7	225	22	9.3	203	2	0.8	99	102
367	15	4.2	352	35	9.5	317	0	0.0	166	151
330	12	3.6	318	28	8.5	290	0	0.0	160	130
1164	45	3.9	1119	116	10.0	1003	4	0.3	529	470

The results of the experiment show that approximately 50 percent of the zygotes died when sooty double-yellow females were crossed to sooty

males. There was exactly a quarter that died in the egg stage. When these eggs were examined under high power, they showed no detectable development. Of the other quarter that failed to reach adult stage, four-fifths died as larvae and the rest as pupae. Eleven of these pupae reached a stage where identification was possible, and, in every case dissected, the individual was found to be a triplo-X female (wild-type in body color). Clearly, then, there were two classes of deaths, corresponding to the two types of aberrations. Since the triplo-X females were identified in the advanced pupae, it seems safe to conclude that the duplication of the X chromosome was lethal in larval and pupal stages, whereas the homozygous deficiency (nullo-X), represented by YY zygotes, died as eggs which showed no signs of development.

None of the triplo-X females emerged as adults in the present experiment, which was contrary to expectation. The control series showed that there was a modifier present in the sooty by sooty matings, where it was responsible for the unduly high mortality in the larval stage. This same modifier was also met with in the Plexate (tables 9 and 10), and Minute-l controls (tables 14 and 15), where the sooty flies were used. Since the triplo-X flies are very inviable and easily affected, it is possible that this modifier was the cause preventing any from reaching the adult stage.

Deficiency and duplication of the fourth chromosome

Non-disjunction of chromosome-IV has been extensively studied both genetically and cytologically by BRIDGES; and since his work has been published in a series of papers (1921, 1922, 1925), much of the detail in these cases will be omitted in the following account.

Haplo-IV

Among the Minutes alluded to in the early part of the paper, one particular type came to be recognized by BRIDGES as of frequent recurrence and characteristic genetical behavior. Among the offspring of these flies, exceptions to the normal distribution of the fourth-group characters occurred, pointing to the conclusion that these exceptions were produced by non-disjunction of the chromosome carrying the fourth group of genes and that the particular type of Minutes consisted of zygotes lacking one member of the fourth chromosome pair.

Cytological examination of the cells of these haplo-IV individuals has shown that one of the small round chromosomes is missing. (Figures published by BRIDGES, 1917 p. 191). Enough mitotic figures have been obtained to make the interpretation positive.

According to BRIDGES, the haplo-IV flies are characterized by low productivity, tardy development, heavy mortality and frequent sterility. Experiments were carried out by the author to determine in a systematic way the approximate percentage of sterile individuals in a given culture of haplo-IV and to locate if possible the internal change causing the sterility.

A single culture from the pair mating of inbred sooty by haplo-IV sooty was used in the experiment. Flies produced by this particular pair were solated every day until the end of the eighth day. This culture altogether produced 70 sooty and 64 haplo-IV sooty individuals (24 females and 40 males). All the haplo-IV individuals, except three, were tested by mating to a Swedish stock. Only virgin females were used in all cases. As controls, ten sooty sibs, five females and five males, taken at different dates of isolation, were also crossed to the same Swedish stock. For every haplo-IV female two Swedish males were used, and for every haplo-IV male two Swedish females were used. After five or six days, the vials were examined. The pairs that produced larvae were classified as fertile; those that failed to do so were dissected and examined microscopically. In case of a haplo-IV female, the female and one of the males were dissected to see if the reproductive apparatus was normal. The male was dissected to find out whether living sperm were present in abundance. Similarly, in case of a haplo-IV male, the male and one of the females were dissected and the other female was mated again to a Swedish male.

It was found that all the control sooty individuals were fertile. Of the 40 haplo-IV males tested twenty were fertile. Eleven produced no offspring and the males died early. Nine others lived longer but were sterile. Eleven were dissected, including two of the early dying males; and all had living sperm in the testes. Upon dissection, the females, to which they had been mated, failed to show the presence of sperm in their spermatheca. The other females, that were remated to Swedish males, all proved fertile. Of the twenty-one females that were tested, thirteen were fertile, eight failed to lay any eggs at all, and six of the eight died early, on about the fifth day. The remaining two were dissected. One of these showed sperm in the spermatheca, and the other did not. Evidently, then, the sterility of the haplo-IV flies in both males and females amounted to approximately 50 percent. The cases of sterility and early death seemed to be scattered at random through the eight-day period of isolation. The sterility was directly associated with short duration of life and the inability to transmit sperm (in the case of haplo-IV males). No deformity of sexual organs was observed in the cases examined.

It has been shown by BRIDGES that haplo-IV flies usually begin to emerge a few days later than their normal sibs. In crosses in which half of the offspring were expected to be haplo-IV, a mean ratio of only 34.5 percent was found. When haplo-IV flies were bred together, a mean ratio of 139 haplo-IV to 100 wild type was obtained, showing that the nullo-IV is lethal. The next problem is to find out at what stage the homozygous deficiency flies die, and to test whether the ratio of the haplo-IV to normal flies would increase under favorable experimental conditions. Two stocks were prepared, one by successive out-crosses of haplo-IV males to sooty females from inbred sooty stock, and the other by selecting and inbreeding the haplo-IV stock itself. Finally, these two lines were crossed to each other in four different combinations, each with a reciprocal cross. The combinations were: (1) haplo-IV by haplo-IV, (2) haplo-IV by normal (diplo-IV), (3) normal (diplo-IV) by haplo-IV and (4) normal (diplo-IV) by normal (diplo-IV), thus furnishing data for the experiments and their controls. A large number of pairs were mated, especially in those series where haplo-IV individuals were used.

Tables 28 to 35 give the essential results.

TABLE 28

Haplo-IV ♀ × *sooty haplo*-IV ♂ (*series 1*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		HAPLO-IV	
									♀	♂	♀	♂
25	17	68.0	8	5	20.0	3	0	0.0	1	0	2	0
117	59	50.2	58	14	11.9	44	2	1.7	7	7	11	17
163	54	33.1	109	16	9.8	93	1	0.6	22	8	32	30
135	46	34.1	89	25	18.5	64	1	0.7	17	7	18	21
117	56	47.8	61	16	13.7	45	1	0.9	6	3	14	21
166	70	42.1	96	31	18.7	65	1	0.6	12	8	19	25
723	302	41.8	421	107	14.7	314	6	0.8	65	33	96	114

TABLE 29

Sooty haplo-IV ♀ × *haplo*-IV ♂ (*contrary cross*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		HAPLO-IV	
									♀	♂	♀	♂
5	3	60.0	2	0	0.0	2	0	0.0	1	0	0	1
8	2	25.0	6	0	0.0	6	0	0.0	3	0	0	3
129	44	34.1	85	7	5.4	78	0	0.0	12	14	19	33
269	78	29.0	191	25	9.3	166	6	2.2	22	29	47	62
164	59	36.0	105	14	9.5	91	2	1.2	14	20	29	26
200	60	30.0	140	8	4.0	132	4	2.0	30	23	41	34
56	18	32.1	38	14	25.0	24	0	0.0	5	5	8	6
831	264	31.7	567	68	8.2	499	12	1.4	87	91	144	165

TABLE 30.
Haplo-IV ♀ × *sooty* ♂ (*series 2*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		HAPLO-IV	
									♀	♂	♀	♂
76	8	10.5	68	11	14.5	57	1	1.3	18	11	16	11
66	7	10.6	59	11	16.7	48	0	0.0	13	9	17	9
89	14	15.7	75	13	14.6	62	1	1.1	18	14	16	13
59	4	6.8	55	8	13.5	47	2	3.4	14	6	10	15
253	33	13.0	220	33	13.0	187	5	2.0	47	41	49	45
134	24	17.9	110	20	14.9	90	1	0.7	23	20	27	19
194	15	7.7	179	18	9.3	161	0	0.0	47	31	41	42
871	105	12.0	766	114	13.1	652	10	1.1	180	132	176	154

TABLE 31
Sooty haplo-IV ♀ × *diplo*-IV ♂ (*contrary cross*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		HAPLO-IV	
									♀	♂	♀	♂
48	11	22.9	37	8	16.7	29	0	0.0	9	8	8	4
57	11	19.3	36	6	10.5	40	0	0.0	6	14	11	9
60	5	8.3	55	8	13.3	47	1	1.6	14	9	14	9
75	20	26.7	55	7	9.3	48	2	2.7	13	10	10	13
86	24	27.9	62	17	19.8	45	1	1.7	15	15	6	8
73	17	23.3	56	9	12.3	47	0	0.0	9	10	16	12
399	88	22.1	311	55	13.8	256	4	1.0	66	66	65	55

TABLE 32
Diplo-IV ♀ × *sooty haplo*-IV ♂ (*series 3*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		HAPLO-IV	
									♀	♂	♀	♂
162	17	10.5	145	25	15.4	120	1	0.6	40	27	21	31
134	16	11.7	118	29	21.6	89	1	0.7	28	21	23	16
49	5	10.2	44	6	13.6	38	0	0.0	8	6	12	12
245	30	12.2	215	27	12.6	188	3	1.2	44	44	39	58
269	39	14.5	230	44	16.3	186	1	0.7	41	40	51	53
859	107	12.5	752	131	15.2	621	6	0.7	161	138	146	170

TABLE 33

Sooty ♀ × *haplo*-IV ♂ (*contrary cross*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+		HAPLO-IV	
									♀	♂	♀	♂
81	5	6.2	76	7	8.5	69	2	2.5	12	14	23	18
113	9	8.0	104	15	13.3	86	1	0.9	21	22	23	22
314	2	0.6	312	12	3.8	300	2	0.6	78	78	65	77
198	4	2.0	194	7	3.5	187	0	0.0	49	39	46	53
163	0	0.0	163	1	0.6	162	1	0.5	42	35	37	47
264	9	3.4	255	7	2.6	248	1	0.4	70	54	55	68
1133	29	2.6	1104	49	4.3	1055	7	0.6	272	242	249	285

TABLE 34

Diplo-IV ♀ × *sooty* ♂ (*series 4, control*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	♀	♂
71	4	5.6	67	8	11.0	59	0	0.0	30	29
64	5	7.8	59	6	9.0	53	1	1.6	29	23
57	4	7.0	53	2	3.5	51	1	1.8	28	22
278	9	3.2	269	13	4.7	256	0	0.0	133	123
240	14	5.8	226	18	7.5	208	2	0.8	104	102
204	7	3.5	197	15	7.3	182	0	0.0	109	73
914	43	4.7	871	62	6.8	809	4	0.4	433	372

TABLE 35

Sooty ♀ × *diplo*-IV ♂ (*contrary cross, control*).

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	♀	♂
61	1	1.6	60	2	3.2	58	0	0.0	39	19
59	2	3.4	57	3	5.1	54	0	0.0	25	29
70	0	0.0	70	8	11.4	62	0	0.0	38	24
160	4	2.5	156	7	4.4	149	0	0.0	76	73
215	5	2.3	210	9	4.2	201	2	0.9	107	92
86	3	3.5	83	2	2.3	81	0	0.0	40	41
651	15	2.3	636	31	4.8	605	2	0.3	335	278

Comparison of the totals for tables 34 and 35 shows that between the two control series there was a difference in general mortality that amounted to 5 percent of the zygotes. In cases where the diplo-IV sisters of the haplo-IV flies were used, there was a higher percentage of mortality in

both egg and larval stages than in cases where the sooty females were used. The same relation seems to be present, but accentuated, in the experimental series. Thus, when sooty females were mated to haplo-IV males, there was no difference in the number of deaths between the experiment and the control (tables 33 and 35). But when the diplo-IV females were crossed to sooty haplo-IV males, there was a decided increase in mortality in both egg and larval stages that amounted to about 20 percent of the zygotes (tables 32 and 33). In both cases, the male was the deficiency-bearing parent, and the difference cannot be attributed to the deficiency. Likewise the haplo-IV female by sooty haplo-IV male cross (table 28) gave a total of about 16 percent more mortality in egg and larval stages than did the contrary cross of sooty haplo-IV female by haplo-IV male (table 29). In both of these cases, all parental flies were haplo-IV and the difference is wholly due to the difference between the origina stock of haplo-IV and the new stock obtained by repeated out-crosses to sooty.

A difference of about 10 percent of zygotic mortality in the egg stage was present between the cross of haplo-IV females and sooty males (table 30) and sooty haplo-IV females by diplo-IV males (table 31). This difference was of opposite direction from that present in the other three pairs of contrary crosses. The four pairs of contrary crosses have therefore given the following differences: 5 percent, 20 percent, 16 percent and −10 percent. These differences cannot be attributed to the haplo-IV, because in all cases the two crosses compared have been the same with respect to the deficiency of the fourth chromosome. Evidently the original stock of haplo-IV differed from the new stock in that it carried a mutant that caused the eggs to be less viable and that caused the larvae that hatched from those eggs to be less viable. A similar but more extreme mutant difference was encountered in the plexus and speck stock (see page 13) where it caused the deaths of fully 75 percent of the zygotes in the egg stage.

If the results of the haplo-IV by diplo-IV crosses (tables 30 and 31) are compared with their reciprocal crosses (tables 32 and 33) evidences of maternal effect are apparent. Contrasting the results shown in table 30 with those in table 33, and then the results in table 31 with those in table 32, it will be found that the maternal effect has given rise to a general mortality in egg and larval stages of about 18 percent and 8 percent of the total number of zygotes. It is probable that deaths have occurred at random in the haplo-IV and the diplo-IV classes.

In the cross of sooty haplo-IV female by haplo-IV male (table 29) there was about 32 percent mortality in the egg stage and 8 percent in the larval

stage. This is an excess of 15 percent over the 25 percent of deaths expected to be due to the nullo-IV. Of this excess about 7 percent is to be attributed as the minimum of mortality observed in the comparable control (table 35) and the remaining 8 percent to the maternal effect of the haplo-IV similar in amount to what was observed to differentiate table 31 and 32. Accordingly it is to be concluded that the nullo-IV zygotes all perished in the egg stage.

A similar conclusion is to be drawn from the cross of haplo-IV female by sooty haplo-IV male (table 28). In this cross the excess of mortality over the expected 25 percent was 32 percent, which is to be attributed to three sources: namely, a maternal effect due to the haplo-IV condition of the mother and a further maternal effect due to the special factor in the original stock, which two effects made a joint mortality of 25 percent as is seen in table 30. The additional 7 percent is then due to the minimum of general mortality as observed in table 35.

The ratio of haplo-IV to diplo-IV was, in both cases, close to the expected 2 : 1, and this fact shows that the distribution of deaths was approximately at random and that the haplo-IV zygotes had probably all reached the adult stage. In supporting the latter conclusion, we may appeal to the evidence obtained particularly in the cross between sooty females and the haplo-IV males (table 33), where the general mortality was exactly the same as the comparable control (table 35) and the ratio between the haplo-IV and the diplo-IV was approximately 1 : 1. However, it was not uncommon to find a haplo-IV individual that had died with only the head emerging from the pupa case, and a good many flies, particularly haplo-IV females, became entangled in the food and died immediately after emergence with their wings still folded. These were carefully dug out from the food or from the pupa cases and classified as adults.

Thus it is easy to see that under the experimental conditions, where larval overcrowding was avoided and care in handling was applied to every individual fly, most of the heterozygous deficiency flies had a better chance to come through than they did under the ordinary culture conditions. The same fact has also been observed in case of the heterozygous Minute-l and of the heterozygous Pale duplication that the viability of the individuals was improved by the improvement of the food conditions. Therefore, in the work on the poorly viable mutants and the so called semi-lethals, it is of cardinal importance that these environmental conditions should be made optimum.

Triplo-IV

Triplo-IV individuals were found by BRIDGES (1921) among the offspring of triploid females. The somatic characters that distinguish triplo-IV individuals from the wild-type are not clean-cut, and the final identification must depend on the genetic ratio in crosses with the fourth group of mutants (eyeless). From the F_1 of triplo-IV by eyeless, triplo-IV flies, according to BRIDGES, could usually be identified by their slightly darker body color and absence of trident pattern, by their narrower, more pointed wing and smaller, smoother eyes. All of these characters are difficult for the untrained observer. When the selected F_1 individuals are backcrossed to eyeless again, the cultures give a ratio of five not-eyeless to one eyeless. Such a result agrees with expectation, if it is assumed that in the F_1 triplo-IV individual, there are 2 fourth-chromosomes carrying not-eyeless genes, and one carrying that for eyeless, and that one not-eyeless gene is dominant over the two eyeless genes whenever they are present together in the backcross flies.

Cytological examination of the germ cells of the females that were classified as being triplo-IV showed that there was present an extra, small, round chromosome (MORGAN, BRIDGES and STURTEVANT 1925).

Triplo-IV has never been found to be lethal. When F_1 triplo-IV flies were bred to each other, the ratio of not-eyeless to eyeless was approximately 26 : 1, which indicated that none of the tetra-IV lived, otherwise the ratio would be 35 : 1, as illustrated in the following table:

$+ + ey$ ♀ × $+ + ey$ ♂

EGGS / SPERM	+ + 1	+ ey 2	+ 2	ey 1
+ + 1	+ + + + 1 dies	+ + + ey 2 dies	+ + + 2	+ + ey 1
+ ey 2	+ ey + + 2 dies	+ ey + ey 4 dies	+ ey + 4	+ ey ey 2
+ 2	+ + + 2	+ + ey 4	+ + 4	+ ey 2
ey 1	ey + + 1	ey + ey 2	ey + 2	ey ey 1

The above table gives only the combinations from a cross of a specific type. There were two other types of crosses that might be expected from triplo-IV individuals of the stock of triplo-IV carried on by Doctor BRIDGES. Formulated by the method used in the above diagram, a condensed table of the expected ratios of eyeless to not-eyeless is shown below:

Cross	*Expected mortality*	*Ratio*
+ + ey × + + ey	25 percent	26 ey : 1+
+ ey ey × + + ey	25 percent	8 ey : 1+
+ ey ey × + ey ey	25 percent	2.4 ey : 1+

Similarily, since one cannot be sure of the identification of the triplo-IV individuals, the parental combinations shown below might also appear, giving the ratios indicated. Through comparison with an index table of this kind one may diagnose the constitution of the parents with some degree of certainty:

Cross	*Mortality*	*Ratio*
+ + ey × + +	0 percent	none eyeless
+ ey ey × + +	0 percent	none eyeless
+ + ey × + ey	0 percent	11 ey : 1+
+ ey ey × + ey	0 percent	3 ey : 1+
+ + ey × ey ey	0 percent	5 ey : 1+
+ ey ey × ey ey	0 percent	1 ey : 1+
+ ey × ey ey	0 percent	1 ey : 1+

The present experiment was planned to find out when the tetra-IV flies die and to what degree the triplo-IV condition has a lethal effect. Through the kindness of Doctor BRIDGES, who picked out a number of the triplo-IV flies, the following four series of crosses were made, triplo-IV by triplo-IV, triplo-IV by eyeless in reciprocal crosses, and eyeless by eyeless. The last series forms the control of the experiment. After the mortality results of each mating had been followed through, the series were checked by comparing the ratios obtained with those of the index tables shown above. The vials were then grouped according to type of parentage and tabulated in tables 36 to 45 inclusive. The presence of triplo-IV individuals among the offspring was also checked.

TABLE 36
$++ey\ ♀ \times ++ey\ ♂$.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	ey♀	ey♂	Total	Percent	+♀	+♂	ey♀	ey♂
201	56	27.8	145	42	20.8	7	5	3	2	1	18	8.9	51	31	1	2

TABLE 37
$+\ ey\ ey\ ♀ \times +\ ey\ ey\ ♂$.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	ey♀	ey♂	Total	Percent	+♀	+♂	ey♀	ey♂
245	37	15.1	208	40	16.3	5	0	3	6	1	15	6.0	53	60	19	21
212	24	11.3	188	40	18.9	15	4	4	0	8	31	14.6	43	49	11	14
457	61	13.3	396	80	17.5	20	4	7	6	9	46	10.0	96	109	30	35

TABLE 38
$++ey\ ♀ \times +\ ey\ ♂$.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	ey♀	ey♂	Total	Percent	+♀	+♂	ey♀	ey♂
342	3	0.9	339	27	7.9	15	7	3	2	0	27	7.9	119	134	22	10
294	4	1.4	290	28	9.5	12	6	5	3	3	29	9.9	109	100	12	12
636	7	1.1	629	55	8.6	27	13	8	5	3	56	8.8	228	234	54	22

TABLE 39

+ *ey ey* ♀ × + *ey* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	*ey*♀	*ey*♂	Total	Percent	+♀	+♂	*ey*♀	*ey*♂
263	9	2.5	353	24	6.6	8	2	1	6	8	25	6.9	122	124	26	32
293	16	5.6	277	20	6.8	15	6	8	9	11	49	12.5	78	86	23	21
160	5	3.1	155	15	9.4	7	2	3	2	3	17	10.6	47	49	13	14
630	18	7.8	212	10	4.3	3	1	4	1	1	10	4.3	87	63	24	18
1045	48	4.6	997	69	6.6	33	11	16	18	23	101	9.7	334	322	86	85

TABLE 40

+ + *ey* ♀ × *ey ey* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	*ey*♀	*ey*♂	Total	Percent	+♀	+♂	*ey*♀	*ey*♂
237	3	1.3	234	7	2.9	2	1	0	0	4	7	2.9	88	87	26	19
318	6	2.1	282	16	5.7	7	0	0	1	1	9	3.2	98	109	22	28
525	9	1.7	516	23	4.4	9	1	0	1	5	16	3.0	186	196	48	47

TABLE 41

+ *ey ey* ♀ × *ey ey* ♀.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	*ey*♀	*ey*♂	Total	Percent	+♀	+♂	*ey*♀	*ey*♂
318	11	3.5	307	93	29.2	0	0	0	1	2	3	0.9	83	25	66	37
288	32	11.4	256	24	8.6	3	0	0	3	6	12	4.3	56	47	63	54
606	43	7.1	563	117	19.3	3	0	0	4	8	15	2.5	139	72	129	91

TABLE 42

$+ ey \; ♀ \times ey \; ey \; ♂$.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	ey♀	ey♂	Total	Percent	+♀	+♂	ey♀	ey♂
173	3	1.7	170	8	4.6	4	2	0	4	8	18	10.4	52	39	26	27
245	10	4.1	235	14	5.7	3	3	5	7	14	32	13.1	42	62	52	33
418	13	3.1	405	22	5.3	7	5	5	11	22	50	12.0	94	101	78	60

TABLE 43

ey ey ♀ × + + *ey* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	ey♀	ey♂	Total	Percent	+♀	♂	ey♀	ey♂
116	2	1.7	114	4	2.4	3	0	0	0	0	3	1.8	49	43	9	6

TABLE 44

ey ey ♀ × + *ey* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED							ADULTS			
						Molts	+♀	+♂	ey♀	ey♂	Total	Percent	+♀	+♂	ey♀	ey♂
237	5	2.1	232	15	6.3	5	0	1	4	1	11	4.6	59	54	39	54
199	9	4.5	190	14	7.0	1	0	0	5	4	10	5.0	42	46	42	36
244	4	1.4	240	22	9.0	3	1	5	13	2	24	9.8	68	44	40	42
220	1	0.4	219	4	1.8	4	0	1	3	1	9	4.1	47	49	57	53
171	8	4.7	163	18	10.5	0	1	1	6	6	14	8.2	37	30	35	29
1071	27	2.5	1044	73	6.8	13	2	8	31	14	68	6.3	253	223	213	214

TABLE 45
ey ey ♀ × *ey ey* ♂.

EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE DIED					ADULTS	
						Molts	♀	♂	Total	Percent	♀	♂
219	3	1.4	216	32	14.6	9	7	7	23	10.5	82	79
125	4	3.2	121	9	7.2	0	2	6	8	6.4	54	58
152	9	5.9	143	2	1.3	4	9	7	20	13.2	63	58
150	7	4.2	143	12	8.0	0	7	4	11	7.3	60	60
192	8	4.2	184	11	5.7	7	14	11	32	16.7	67	74
838	31	3.7	807	66	7.9	20	39	35	94	11.2	326	321

As seen in the control and in each experiment, the mortality of the eyeless flies was very high. It was especially marked in the pupal stage. When eyeless was mated to eyeless, the general mortality went up to a total of 22.8 percent of the number of zygotes (table 45). It is probable that the high death rate in the eyeless by heterozygous eyeless series (tables 42 and 44) and in one of the triplo-IV by eyeless series (table 41) was due to the presence of a large number of eyeless flies that were inviable.

Results shown in tables 40 and 43 from reciprocal crosses of "+ + ey" by eyeless indicate that triplo-IV as such was not lethal, as deaths occurring in these series were even lower than in the controls. No maternal effect or late emergence has been observed in either the triplo-IV or the eyeless flies.

Tables 36 and 37 give the results of the triplo-IV by triplo-IV crosses. Tables 38 and 39 give the results of triplo-IV by heterozygous eyeless which, therefore, may serve as controls of the preceding experimental matings. From these tables it is seen that while no difference in percentage of deaths could be found in the pupal stage, there were about 30 to 40 percent more zygotes that died as eggs and larvae in the experiments than in the controls. The conclusion seems evident, then, that the zygote with tetra-IV constitution died mostly in the egg stage, but also in the larval stage.

Summary of chromosomal aberrations

1. In the second part of the present work, it has been demonstrated that both nullo-X and nullo-IV died as eggs and the zygote with YY constitution showed no sign of development. The results confirm what had been observed in the previous analyses. We may safely conclude, therefore, that zygotes with homozygous deficiencies (nullo-X, nullo-IV,

Notch male, Px/Px, Ml/Ml, P_{II}/P_{II} and perhaps $P_{II}\ P_{III}/P_{II}\ P_{III}$) all die in a more or less clean-cut way in one of the egg stages.

2. The heterozygous deficiencies (haplo-IV, $Ml/+$) have mortality little higher than normals, especially when the male is the deficiency-bearing parent. In some cases, the viability of the heterozygous deficiency flies (Notch female, $Px/+$) is almost as good as the normals. The larger deficiencies, however, ($P_{II}/+$ and its equivalents) owing to the loss of a greater number of genes are lethal in the larval stage.

3. The corresponding homozygous duplication case, as illustrated in tetra-IV, dies later than the homozygous deficiency, some lasting into the larval stage. Comparing with the results of the sectional duplications, we find homozygous Pale duplication (P_{III}/P_{III}) dying still later, some even attaining full larval growth (molt-stage).

4. In regard to the heterozygous duplication cases, triplo-IV may be contrasted with triplo-X. In the former scarcely any lethal effect was observed, and in the latter all died in late larval and pupal periods.

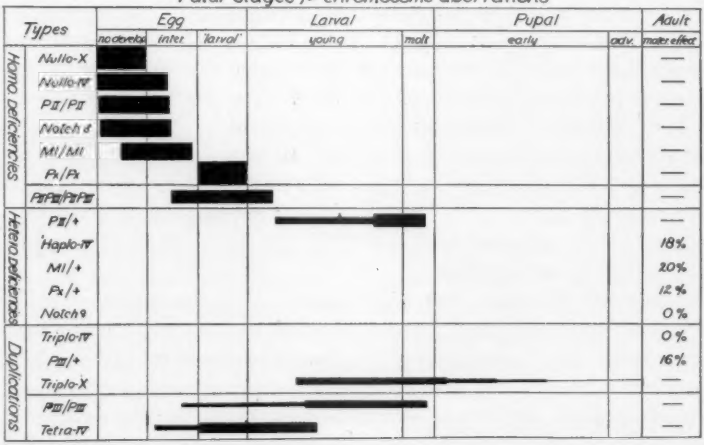

FIGURE 4.

There is, of course, a great difference in the number of genes involved in these two cases; and on this ground the difference in the fate of the two types of zygote might be expected. In between these two extremes, we may place the sectional duplication ($P_{III}/+$). Under favorable conditions, all the heterozygous Pale duplication zygotes lived, but usually the viability was lower than normal. There are seven units involved in

the Pale duplication and the section is probably longer than the length of the whole fourth chromosome. Clearly, then, the difference in lethal effects in these cases might be attributable to the difference in the number of genes involved. In this respect heterozygous duplications are comparable to heterozygous deficiencies.

The lethal stages for different types of chromosome aberrations are represented in figure 4.

Retarded stages in haplo-IV flies

In stock cultures, the haplo-IV flies generally begin to emerge two to three days later than their normal sibs. The same delay was also observed, though of shorter duration, under the experimental conditions. Such delay evidently could not be entirely due to the environmental factors, but rather was mainly due to reactions of the aberrant group of chromosomes, which presumably determined the rate of development. The following experiment was carried out in an attempt to locate the specific stage or stages at which retardation occurs.

Two of the previous series dealing with haplo-IV were repeated, namely, sooty females by haplo-IV males and sooty females by diplo-IV males from the haplo-IV stock, the last furnishing the control. Eggs were isolated at six-hour intervals for five days after the first batch of eggs were laid. The same six-hour intervals were used in following the subsequent developmental stages of the flies. All records of the counts were carefully kept on a chart, so that practically every individual fly was given consideration in regard to the time at which it entered each of its different stages. Freshly prepared food was put into each vial with a counted number of larvae (about 15–20).

As indicated elsewhere, the critical feature of an experiment of this kind is the proper control of the larval period, particularly the number of larvae in each vial. A condition of overcrowding would interfere with the proper development of the larvae and, at the other extreme, too few larvae isolated in a given amount of food would allow the food to go bad. In case only a few larvae were isolated at a particular time, a smaller amount of food was used instead of the standard amount (see appendix). Larvae of the stock of eyeless[4] were mixed in with the isolated ones. In these eyeless[4] flies the head is small with complete absence of eyes and the adults can, therefore, be separated from the experimental flies when both emerge. Also, because of the deformity of the eyeless[4] fly, the anterior part of its pupa case is more or less flattened and pointed and, accordingly, the distinction could be made even in the pupal stage. The eyeless[4]

larvae used were generally two to three days older and they usually pupated earlier than the larvae of the experiment.

The females were mated about two to three days after their emergence and the first batches of eggs were produced within eight hours after the time of mating. Eggs produced by four pairs of sooty by haplo-IV were treated together without following any particular pair. The same was done for the control series including three pairs of sooty by diplo-IV brothers of the haplo-IV. The first five days of the experiment were employed in isolating eggs and larvae; at the end of the fifth day, the isolation of the pupae and adults took up all the time. The results of the whole experiment, as shown in tables 46 to 50 inclusive, are given in summaries.

Tables 46 to 50 bring out the following facts:

1. The retardation of the haplo-IV flies occurred in all three developmental stages, but especially in the larval stage. If the length of time involved in the delay is expressed in percentage of each period by dividing the difference of the two means (that is, between the means of the diplo-IV and the haplo-IV individuals) in the sooty by haplo-IV series by the mean of the control series, the results are as follows: In the egg stage, the haplo-IV flies were delayed 2.9 percent of the mean egg period; in the larval stage, 26.4 percent of the mean larval period; and in the pupal stage, 5.2 percent of the mean pupal period.

2. A bi-modal distribution was shown in the larval period in the sooty by haplo-IV cross (table 47). On the average, haplo-IV individuals pupated 22.3 hours later than their diplo-IV sibs. Since all the larvae were isolated at the same time and treated alike under well-controlled conditions, the delay means that the haplo-IV flies have a rate of development which is inherently slower than that of the diplo-IV. In both the egg and pupal stages (tables 46 and 48) the delay was evident, and there was hardly any differential environmental influence that could affect these periods under the experimental conditions.

3. Another bi-modal distribution was shown in the pupal period of both control and experimental series, representing the difference between male and females in the length of pupal period (table 49). As far as the present data go, careful analysis failed to bring out correlated differences before the pupal period, but this does not necessarily mean that there was no difference between males and females in the larval period, for it might be possible to detect such a difference by using smaller intervals of isolation. It does mean, however. that the difference in the rate of growth and differentiation between males and females is obviously striking in the

TABLE 46

Hatching of eggs in hours after eggs were laid.

SERIES	CLASS	EGGS	6	12	18	24	30	36	42	48	LARVAE	MEAN	ST. DEV.
Control	Wild-type	578	2	1	116	441	9	3			572	22.86±0.06	2.94±0.06
e^s ♀ × Hiv ♂	Diplo-IV	492	1	3	25	157	20		1		207	23.68±0.17	3.60±0.11
	Haplo-IV				28	184	29	3	1	1	246	24.34±0.16	3.72±0.11

TABLE 47

Pupation of larvae in hours after eggs hatched.

SERIES	CLASS	LARVAE	60	66	72	78	84	90	96	102	108	114	120	126	PUPAE	MEAN	ST. DEV.
Control	Wild-type	572	1	0	50	149	184	88	34	17	14	3	3	1	544	84.54±0.26	8.94±0.20
e^s ♀ × Hiv ♂	Diplo-IV	207	2	5	79	75	32	8	2	2					205	77.04±0.36	6.24±0.24
	Haplo-IV	246				2	13	29	79	68	35	15	3		244	99.30±0.33	7.80±0.24

TABLE 48

Emergence of pupae in hours after pupation.

SERIES	CLASS	PUPAE	78	84	90	96	102	108	114	120	ADULTS	MEAN	ST. DEV.
Control	Wild-type	544	4	51	158	135	90	63	9	2	512	95.76±0.23	7.74±0.16
e^s ♀ × Hiv ♂	Diplo-IV	205	2	9	51	60	52	10		3	187	96.28±0.34	6.84±0.24
	Haplo-IV	244		2	16	65	68	42	8	6	207	101.22±0.33	7.02±0.23

TABLE 49

Emergence of pupae in hours after pupation, according to sex.

SERIES	CLASS	SEX	78	84	90	96	102	108	114	120	ADULTS	MEAN	ST. DEV.
Control	Wild-type	♀	4	46	111	83	21	6			271	91.98±0.24	5.80±0.18
		♂		5	47	52	69	57	9	2	241	100.20±0.32	7.42±0.23
e^s ♀ × Hiv ♂	Diplo-IV	♀	2	8	37	25	13			1	86	92.06±0.46	6.30±0.32
		♂		1	14	35	39	10		2	101	99.30±0.47	7.02±0.33
	Haplo-IV	♀		2	15	47	36	14		2	116	98.76±0.39	6.18±0.27
		♂			1	18	32	28	8	4	91	104.32±0.48	6.81±0.34

TABLE 50

Summaries.

SERIES	EGGS	EGGS DIED	PERCENT	LARVAE	LARVAE DIED	PERCENT	PUPAE	PUPAE DIED	PERCENT	+ HIV			
										♀	♂	♀	♂
Control	578	6	1.0	572	28	4.9	544	32	5.5	271	241	..	..
e^s ♀ × Hiv ♂	492	11	2.2	481	28	5.7	453	59	12.0	86	101	116	91

pupal period. Such difference, as recently shown by BLISS (1926), are appreciable as early as the prepupal stage.

4. We have seen that there was a difference between diplo-IV and haplo-IV individuals in the time of emergence; the carriers of the deficient set of chromosomes were retarded particularly in the larval period. We have also seen that a like time-difference was present in the males and females; the males were retarded (or else the females were accelerated) particularly in the pupal period. We may consider the XY-chromosome combination in the male as comparable to a deficiency of one X chromosome (haplo-X type). Thus, the alteration of the balance between two sets of genes is directly responsible for the retarding process in both cases; and the genes in different chromosomes may be further assumed to exert this influence specifically in the different developmental stages of the flies.

5. It is known that there is a difference in size between males and females, the males being smaller. Such difference in size also exists between the diplo-IV and haplo-IV flies. Since the size of the adult is predetermined in the pupa, and large pupae in turn must come from large larvae, the size of the larvae, then, is limiting factor of the size of the flies. If this is correct, we can easily see that the influence of the unbalanced set of genes manifests itself not only in the retardation of the rate of reactions, but also in the product formed by the reactions.

The correlation between the length of larval and pupal period

Further analysis of the above data, particularly of the control series, wherein sooty females were crossed to diplo-IV males from the haplo-IV stock showed a relation that presents three aspects, namely, (1) a change in means of lengths of larval and pupal periods with progressive age of the parents, (2) a negative correlation between lengths of larval and pupal periods and (3) a resultant constancy of the total length of time for development.

TABLE 51

Change in means of length of larval and pupal periods with progressive age of parents.

TIME AFTER ISOLATION IN DAYS	MEAN LENGTH OF LARVAL PERIOD IN HOURS	MEAN LENGTH OF PUPAL PERIOD IN HOURS	TOTAL TIME IN HOURS	NUMBER OF PUPAE	NUMBER OF ADULTS
1	91.92±0.82	88.32±0.24	180.24	124	116
2	82.08±0.54	92.34±0.60	174.42	47	44
3	84.72±0.66	94.26±0.48	178.98	67	66
4	81.24±0.30	97.98±0.39	179.22	151	144
5	82.62±0.42	101.04±0.34	183.66	153	140
Mean	84.54±0.26	95.76±0.23	180.30	542	510

As shown in table 51, there was a tendency to shifting in the means from a longer larval period in the early batches of eggs to a shorter larval period in the later batches. Conversely, the data on the pupal period show that a more marked shift of the means in the opposite direction occurred. There was thus a definite age-change that caused a shorter larval period and a correspondingly longer pupal period in the offspring as the parents became older. Such an age-change has also been met with in BLISS'S (1926) work on the relation between temperature and the development of the fly in the prepupal stage. As to the cause of the age-change, we can at best only conjecture in the absence of further evidence. Results on the delayed period of haplo-IV flies indicated that the length of the larval period depended on the balance of the genes in the zygote, and on the nature of the cytoplasm of the egg.

Table 51 also shows a negative correlation between the time spent in the larval period and that in the pupal period. With the shortening of the larval period there was a corresponding lengthening of the pupal period, which made the total length of the periods approximately equal. Taking every adult into consideration, the following correlation table was made:

Hours in pupal stage	72	78	84	90	96	102	108	114	120	
	49	142	178	77	33	15	13	1	2	510
114	2	2	4		1					9
108	8	9	32	7	5	2				63
102	10	45	18	14	2		1			90
96	18	48	34	24	9	2				135
90	10	29	66	20	15	9	8		1	158
84	1	9	21	12	1	2	3	1	1	51
78			3				1			4

Hours in larval stage

The coefficient of correlation is $-.22 \pm .03$, which is about seven times the probable error. It is therefore significant despite the uncontrollable factors, such as the relative freshness of food and its degree of fermentation. Another disturbance resulted from the excess of the flies that emerged at daybreak.

Since the amount of decrease in the larval period (about 10 hours), as the parent changed in age, was approximately the same as the amount

by which the pupal period increased (about 13 hours), there was almost no change in the total length of time spent in development. Presumably there is some reaction determining the end of development that continues through the larval and pupal stages alike and if the larval stage is unduly short, the pupal period must be correspondingly longer before the end-product is reached.

PICTET (1902), in one of the early papers on insect physiology, demonstrated the presence of a correlation between larval and pupal periods in moths. By feeding caterpillars with different kinds of food (walnut leaves or others), he could lengthen or shorten the larval period at will under experimental conditions. Those food plants that gave insufficient nourishment prolonged the caterpillar stage; but this led to a shortening of the pupal stage. Conversely, an abundant nourishment accelerated the caterpillar stage with a corresponding retardation in the pupal stage. This highly significant discovery has never been confirmed in other classes of insects.

As a matter of fact, both NORTHOP (1917) and BAUMBERGER (1919), who have worked on the duration of these stages in Drosophila in relation to food conditions, have reached entirely different conclusions. Both agree that there is no correlation between larval and pupal periods in Drosophila. In NORTHROP'S work, we find an entirely different technique from that used here. He raised his flies in 120 cc Erlenmeyer flasks under aseptic conditions. "Flies of both sexes were put in and allowed to remain 15 hours, during which time a number of eggs were laid." The flies were then removed from the flasks, and the eggs in the flasks were placed in a 30°C incubator. For the sake of comparison, NORTHROP'S results (Jour. Biol. Chem. 30: p. 183, table II) are shown below:

RATIO, BANANA TO YEAST	TIME FROM EGG TO PUPAE DAYS	TIME AS PUPAE IN DAYS	TOTAL TIME
0	4.12±0.04	3.43±0.05	7.55
1	3.72±0.02	3.62±0.07	7.32
2	3.60±0.01	3.68±0.04	7.28
4	3.74±0.02	3.54±0.05	7.28
8	4.33±0.03	3.49±0.05	7.82
16	4.75±0.03	3.23±0.10	7.98
32	5.33±0.08	3.45±0.15	8.78
64	6.68±0.16	irregular;	
128	8.30±0.20	flies abnormal	
256	9.00±0.20		
	14–25		

The part of the series from "0" to "16" inclusive in the above table shows a slight correlation between larval and pupal period in that with each relative increase of the length of the larval period, there was a corresponding decrease of pupal period and *vice versa*. The changes in pupal time were slight in amount and very doubtfully significant as judged by the probable errors, but almost certainly mean that the uncontrolled factors were greater in effectiveness than the controlled factor of ratio of banana to yeast. These uncontrolled factors were, therefore, great enough in amount to nearly swamp any larval-pupal correlation.

However, the larval-pupal correlation shown in the results of table 51 were due to a specific agency, namely, change in the age of the parents. In NORTHROP'S work this age factor may have been nearly excluded by the use of parents of slight range in age, and by allowing them to lay eggs for only a short period (15 hours). Perhaps the differences in larval period induced by relative amounts of food affect a developmental process different from the one affected by the change in the age of the parents, and in that case there need be no correlation between larval and pupal durations.

DISCUSSION AND CONCLUSION

The data from NORTHROP'S paper (1917) shown in the preceding page are in rough agreement with another phenomenon that has been brought out in the present work. By adding together lengths of both the larval and the pupal periods, a number that is more constant is obtained. This relative constancy in the total time of development may be correlated with differences in the amount of food taken in during larval period. The individual with a longer larval period will take in more food and, consequently, its pupal stage will be accelerated, and *vice versa*. However, there may be other factors that must not be overlooked. BAUMBERGER (1919) showed that there is a tendency for the larva to pupate after a certain length of time ("periodicity") whether it reaches the maximum size before this period or is still undersized. There seems to be something more fundamental than merely the total energy of the food that underlies the phenomenon of the constancy of the developmental rate. LEOB and NORTHROP (1917) through their work on the duration of life of Drosophila, have concluded that the length of the larval and pupal as well as the adult period is determined "by the formation (or by the disappearance of) a edfinite substance." Later PEARL, PARKER and GONZALEZ (1921, 1922, 1923), have gone over somewhat similar ground as LEOB and NORTHROP and have shown "that under constant environmental conditions, definite

degrees of duration of life are associated with extreme precision and exactness with the presence and absence of certain genes in the chromosomes."

Genetic and physiological evidence of another sort may also help to throw some light on the question. It has been repeatedly shown that the action of some of the genes affecting the adult organs could be influenced by environmental factors during the developmental stages of the fly. DEXTER (1914) showed that more beaded wings were produced in a wet than in a dry culture, and more in an alkaline than in an acid culture. Supernumerary bristles were decreased by a scanty food supply, as MACDOWELL (1915) maintained. MORGAN (1915) has also shown that the development of the abnormal abdomen through a gene located in the X chromosome was influenced and even controlled entirely by the moisture condition of the food at a critical period during the first few days of the larval life. A gene also in the X chromosome causing a reduplication of legs in the adult was shown to be influenced by temperature. From Miss HOGE's data (1915), it is possible to ascertain that the low temperature was effective chiefly during the early larval life. The character for vestigial wings, as shown by ROBERTS (1918) and others, is modifiable by raising the temperature to about 30°C; the effective period is probably late in larval life or else just after pupation.

Probably the most careful work done along this line is that of SEYSTER (1919) and KRAFKA (1920) on the effect of temperature on the development of the facet number in the bar-eye character. KRAFKA showed that the duration of the effective period in percentage of the total is the same in both high and low temperature experiments. The effective period begins when about 32 percent of the time from hatching to emergence has passed, and ends when about 45 percent of that time has passed; in other words, it is only during a definite and relatively short period of larval life. The significant fact established by these experiments is that the length of a particular phase of development is proportional to that of the total phase, and that a process such as that which determines the facet number of the eye has been carried on in a highly specific stage during the larval period.

These facts clearly indicate that practically all the adult organs have certain phases of their development as far back as the early larval stage, and that the process is a continuous one. This view finds an abundance of support in the evidence offered by the study of embryology of the fly and related insects. According to LOWNE (1892) IMMS (1924) and many others, the usual time of the appearance of imaginal discs is during the

early larval stage, and in higher Diptera they were already evident in the embryo. LOWNE showed that in the "nymphoid" stage of the early embryo of the blow fly, many primordia that later develop into adult organs are present, including appendages of the head, the wing, the leg, genitalia and so on. These structures lie relatively dormant or "retrogress," as LOWNE puts it, as soon as the larval tissue is developed in the later stage of the embryo. Regarding the internal organs, such as the dorsal organ, central nervous system and genital apparatus, the same structures, in the opinion of IMMS, persist from embryo to adult uninterrupted throughout the whole period of growth and differentiation. Direct evidence of this kind in Drosophila has also recently been brought forward from a preparation of Mr. CHEN in the COLUMBIA Laboratory. The sections of late larvae yield unmistakable evidence of the presence of the wing and leg discs near the brain region.

After pupation and during the prepupal stage, a profound change takes place. The imaginal discs grow and expand at the expense of the larval tissues, which undergo a complete dissolution. If we call the process relating to the larval growth and its final dissolution a "larval process," and those relating to the growth and differentiation of the imaginal discs an "imaginal process," we may, for the sake of discussion, distinguish two main processes in the life cycle of the fly. If we further assume that the imaginal process has a rather constant rate of development, under constant temperature and other environmental conditions, and is to a certain limited extent independent of the larval process, we may at least suggest a way to explain some of the present problems.

During the larval stage, we may think of these two processes as running side by side; the imaginal process necessarily depends on the larval process for its growth and expansion. We might conceive also that there must have been a constant adjustment between these two processes in order that the larval stage may pass normally, and that the larva may pupate, otherwise abnormalities would occur, including early mortality of some larvae and others that can only go through a molt-stage.

The results of the present experiments demonstrate that the larval stage in the development of the fly is particularly sensitive to environmental conditions, and that as soon as the individual passes the threshold of pupation, it is highly probable that it will reach the adult stage. These observed facts are significant in the light of the foregoing postulate. NORTHROP's (1917) data show that by prolonging the larval period through malnutrition, he obtained many abnormal pupae and adults even if the larval period was stretched only one day beyond the normal

length (see NORTHROP's table on p. 48). On the other hand, by the use of low temperature, he could prolong the larval period as long as 17.8 days at 15°C without interfering with the normal process of metamorphosis. These facts may be taken to mean that temperature affects both imaginal and larval process alike, hence no matter how long the larval process is stretched by low temperature, the imaginal process will adjust itself normally; whereas we may suppose that by starvation one of the two is retarded more than the other; and, consequently, one may be so far behind the other that no adjustment between the two is possible. BAUMBERGER (1919) observed that after a larva had been retarded 26 days, it could be induced to pupate by changing the food. This seems to indicate that it is the imaginal process that is delayed during starvation, since the latter must derive its substance from the larva. The implication of the present hypothesis is that the imaginal process is the one of the two that has a constant rate of growth under constant temperature at the expense of the larval process, whose duration may be more flexible.

The haplo-IV case may furnish a key to the whole situation in understanding the specific effect of chromosome aberrations on development. The evidence shows that the loss of a fourth chromosome retards the developmental process in all three stages, particularly in the larval stage. It clearly indicates that essentially the imaginal process is slowed down on account of the changed genic balance. This is evident when we recall that the time of delay in percentage of the mean egg and pupal period is about the same; and in both of these stages we are dealing mainly with the imaginal process. This retardation of the imaginal process may be considered as a typical effect of some of the chromosome aberrations or similar chromosome alterations in the early embryonic life.

On the assumption that the genes are fairly evenly distributed throughout the chromosomes, we would expect that the loss of one of the small fourth chromosomes would involve, comparatively speaking, only a limited number of genes, and the genes in the homologous chromosome might still bring development to completion. In the loss of a larger piece of chromosome, the chances are that a larger number of genes are lost and such a loss might be so great that the allelomorphic genes would not be able to take care of the proper balance. Consequently the imaginal process is retarded to such a degree that no pupation is possible. The death of heterozygous Pale deficiency ($P_{II}/+$) zygotes probably exemplifies this case.

In considering the duplication cases, we may start from the heterozygous Pale duplication ($P_{III}/+$), in which, although all the individuals did come through, they showed a delay relative to their normal sibs.

Again in the triplo-X, we have seen that when some of the individuals did reach the adult stage, they were much later than the rest; and from the present experiment, it is easy to show that the pupae that died (presumably triplo-X females) were also retarded in the time of pupation. It follows, then, that heterozygous duplication cases are like deficiencies in that the effect of aberration expresses itself in a retardation of the imaginal process. When this is carried further in the homozygous duplication cases, the final effect would be so fatal as to be comparable to the heterozygous deficiencies involving large pieces of chromosome. It seems to be a fact that the heterozygous duplication has a smaller effect than the heterozygous deficiency for an equal length of chromosome involving the same set of genes (P_{II} *versus* P_{III}, haplo-IV *versus* triplo-IV).

We may place the homozygous deficiencies under a slightly different category from the preceding cases. We may look upon the heterozygous deficiencies and duplications generally as involving, essentially, a quantitative change; and the homozygous deficiencies, both a quantitative and a qualitative change of the chromosomes. It is clear that when both sections from a pair of homologous chromosomes, or both of the two homologous chromosomes themselves, are lost, there are eliminated from the chromosome complex a few, or a number of genes, that are now entirely unrepresented. The trend of the series of reactions that follows the event of fertilization must stop as soon as the specific substances produced by those missing genes are called for. We would expect, under the circumstances, the different effects would be somewhat clear-cut and manifest early in the developmental stage, which is the case.

In most of the results of the present work, complicating factors like maternal effect have unexpectedly increased the percentage of mortality in the egg stage and have so far intermixed with the effects of deficiencies that it is impractical at the present time to completely unravel these two sets of influences from the data. In two cases at least, namely, the homozygous Plexate and nullo-X, it has been shown that the eggs that died as a result of homozygous deficiencies have their embryonic development stopped at a very specific stage. Such a specificity associated with the loss of a particular set of genes can hardly be explained in any other light than the hypothesis herein postulated.

SUMMARY

1. The developmental stages of flies carrying aberrant groupings of chromosomes have been followed by counting the eggs, larvae, pupae and adults successively in an effort to determine the precise stage at which

the lethal effects of the different types of chromosome aberrations are produced.

2. It is found that in cases where sections of chromosomes are involved in the aberration, the homozygous deficiencies in the autosomes, such as Plexate, Minute-l and P_{II}, and deficiency in the X chromosome in the male, such as the Notch males, are all lethal in the egg stage. The heterozygous deficiencies, on the other hand, may not have any appreciable effect on the continuity of development of flies unless the section involved is rather long, as in the Pale deficiency case, in which the zygote dies in the larval and molt stage. In sectional duplication cases, the heterozygous P_{III} is not lethal, but the homozygous condition of the same allows no zygote to reach the stage of pupation.

3. In cases where whole chromosomes are involved, such as nondisjunction of the X chromosome and of the fourth chromosome, the effects are comparable with the preceding cases; the homozygous deficiencies of the chromosomes, as seen in the nullo-X and nullo-IV zygote, die as eggs. The heterozygous deficiency, such as in haplo-IV, all comes through under favorable environmental conditions. In the present experiment, the triplo-X condition is shown to be lethal in larval and pupal stages, while the triplo-IV hardly has any mortality whatever. The tetra-IV flies, however, all die, probably in egg and early larval stages.

4. The haplo-IV furnished material for an intensive analysis of the retarded stages of the haplo-IV flies. By six-hour intervals of isolation, it was demonstrated that these aberrant flies are delayed in all three developmental stages, and especially in the larval stage.

5. A careful analysis of the data in the control series of the foregoing experiment with six-hour intervals of isolation led to the discovery of the phenomenon of age-change with correlation of larval and pupal periods.

6. An attempt is made to interpret the results in a consistent way under the fundamental assumption of genic balance. Any disturbance of such a balance would result in a retardation of the developmental process more or less fatal to the flies.

The work herein reported was begun at COLUMBIA UNIVERSITY Laboratory in the spring of 1924 and completed two years later. The author is especially indebted to Professor T. H. MORGAN and Doctor C. B. BRIDGES for supervising the work and correcting the English text.

APPENDIX

DESCRIPTION OF THE METHODS

Control of the stock: Before starting an experiment, the stocks to be used were purified by careful inbreeding. As a rule, brother and sister

pair matings were made; and inbreeding was carried on for four and five generations; selections were made of the pairs that gave the largest and healthiest families in each generation. A mutant stock called "sooty" having good viability and closely inbred for many generations has been kept on hand. In some cases stocks were prepared by crossing the mutants in question to this sooty stock, and the F_1 females from such a cross were again crossed to sooty males and so on for several generations, by the end of which time the chromosome composition of the stock thus prepared would be highly similar to that of the sooty flies. These precautions were taken in the hope that the different stocks would give more comparable results since they would be similar in the modifiers present in each. Again, in the experiments, two distinct lines have been brought together so that, should any recessive genes that are injurious not be eliminated by inbreeding, they might be prevented from interfering with the specific effects of the particular chromosome situations. By heterosis it was further hoped that the vigor of the progeny might be increased.

Control of the experiment: Each series of matings in an experiment was controlled by matings with normal constitution of the chromosomes, and therefore giving normal development. Double controls and reciprocal crosses were used in all cases to check up maternal effects and the like. Excepting in three cases, the experiments were repeated for a second time to insure reliability of the data.

Control of the environment: All experiments were done in 4×1 inch vials (specimen tubes). Flies were raised from eggs to adult stage in a 24°C constant temperature incubator on standardized banana food. With the exception of the Notch deficiency, strictly pair matings were used. Virgin females were isolated as soon as they emerged from the pupa cases by emptying the stock bottles every two hours. The flies were kept isolated for one or two days after their emergence before they were mated. This was done for a twofold purpose: In the first place, by the end of the second or the third day enough virgin flies could be secured to start all matings at the same time. This synchronous start was thought advisable, if all the pairs were to be subject to identical environmental conditions. And, secondly, since the flies generally would not start laying until a little more than 48 hours after their emergence, it would save at least a day's labor in looking after the vials.

Control of the counts: In order to obtain accurate egg counts, the following method was adopted. Glass "slides" (3×1) were cut in two, lengthwise, to make narrow strips, which would fit in the vials. On such a strip of glass was placed a piece of green blotting paper of smaller dimen-

sion so that no paper would come in contact with the wall of the vial. The paper was thoroughly soaked in a yeast culture maintained in banana water mixture. The right degree of fermentation in the banana juice is essential in inducing the flies to lay eggs on the paper. Then a thin layer of thoroughly cooked banana agar food was carefully spread on top of the paper. This preparation would furnish the flies with both food and moisture to lay eggs on. The eggs, being white, would show up easily on the colored paper. The use of this kind of blotting paper has also another advantage. Owing to the thickness, it holds sufficient moisture and it is tough enough to last through the subsequent handling.

Occasionally eggs were counted twice a day, but generally the eggs accumulated during 24 hours. As soon as the eggs were laid, the paper with eggs on it was removed from the vial, and a new paper similarly prepared was put in with as little disturbance to the flies as possible. The change was undertaken at least once a day. The transfers may be continued for eight to ten days, depending on the number of eggs produced and other factors. No pair was carried beyond the tenth day, however.

The eggs were counted under a binocular and recorded. The paper with the eggs on it was then transferred to fresh food in another vial. After two days, when the eggs had presumably all hatched, the paper was taken out of the vial and again examined and the number of eggs left unhatched was counted and recorded. These were presumably the number of flies that died as eggs. To safeguard the results, these unhatched eggs were again given another two days in a new vial and then again counted; the second count went on the record as final.

After the larvae pupated, another count was taken of the number of pupae. The difference between the number of eggs hatched and the number of pupae recorded represents the number of flies that died as larvae. This method of deduction, instead of counting larvae directly, was of decided advantage, both in the labor saved and in the accuracy of the counts. Those who have worked with larvae and have handled them in large numbers will understand how difficult it is to obtain accurate count of the larvae themselves. Handling them might also interfere with their normal development.

Finally, when the adult flies emerged from the pupa cases, the last count was taken. They were at the same time classified in order to determine what kind of flies had died. If a dead imago was fully developed, the pupa case was opened and the nymph classified.

Of the three stages, the eggs are least influenced by food factors, and the larvae most. This is particularly true with factors such as moisture,

fermentation and overcrowding. It is essential, therefore, that these conditions must be optimum. The food was always fresh. No yeast was added aside from what was carried in with the moistened blotting paper at the time when the eggs were transferred to the vial. This procedure would be expected to keep fermentation and acidity of the food under proper control. To avoid overcrowding, the number of flies were standardized in the following way: Vials were made up to contain each about a teaspoonful of food. No more than twenty-five larvae were present in each vial. In cases where one day's output of eggs was less than twenty-five, two day's counts would be put together in the same vial.

LITERATURE CITED

BAUMBERGER, J. P., 1919 A nutritive study of insects with special reference to microorganisms and their substrata. Jour. Exp, Zool. **28**: 1–81.

BLISS, I. C., 1926 Temperature characteristics for prepupal development in Drosophila. Jour. Gen. Physiol. **9**: 467–495.

BONNIER, G., 1924 Contributions to the knowledge of intra- and interspecific relationship in Drosophila. Acta Zool. **5**: 1–122.

1926 Note on so-called vermilion duplication. Hereditas **7**: 229–232.

BRIDGES, C. B., 1913 Non-disjunction of the sex-chromosome of Drosophila. Jour. Exp. Zool. **15**: 587–606.

1917 Deficiency. Genetics **2**: 445–465.

1919 Duplication. Anat. Rec. **15**: 357.

1919 Vermilion-deficiency. Jour. Gen. Physiol. **1**: 645–656.

1921 Genetical and cytological proof of non-disjunction of the fourth chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **7**: 186–192.

1921 Triploid intersexes in *Drosophila melanogaster*. Science N.S. **54**: 252–254.

1923 Aberrations in chromosome materials. Eugenics, Genetics and the Family **1**: 76–81.

BRIDGES, C. B., and MORGAN, T. H., 1919 The second-chromosome group of mutant characters. Carnegie Inst. Washington Publ. **278**: 123–304.

1923 The third-chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. **327**: 251 pp.

DEXTER, J. S., 1914 The analysis of a case of continuous variation in Drosophila by a study of its linkage relations. Amer. Nat. **48**: 712–758.

GONZALEZ, B. M., 1923 Experimental studies on the duration of life. VIII. The influence upon duration of life of certain mutant genes of Drosophila. Amer. Nat. **57**: 289–325.

HOGE, M. A., 1915 The influence of temperature on the development of a Mendelian character. Jour. Exp. Zool. **18**: 241.

IMMS, A. D., 1924 A general text book of entomology. 698 pp. New York: E. P. Dutton and Co.

KRAFKA, J., Jr., 1920 The effect of temperature upon facet number in the bar-eyed mutant of Drosophila. Jour. Gen. Physiol. **2**: 409–464.

LOEB, J., and NORTHROP, J. H., 1917 On the influence of food and temperature on the duration of life. Jour. Biol. Chem. **32**: 103–121.

LOWNE, B. T., 1890 The Blow fly. 778 pp. Cavendish Sq. W. London: R. H. Porter.

MOHR, O. L., 1919 Character changes caused by mutation of an entire region of a chromosome in Drosophila. Genetics **4**: 375–282.

MOHR, O. L., 1922 Cases of mimic mutations and secondary mutations in the X chromosome of *Drosophila melanogaster*. Zeitschr. indukt. Abstamm. u. Vererb. **28**: 1–22.

1923 A genetic and cytological analysis of section deficiency involving four units of the X chromosome in *Drosophila melanogaster*. Zeitschr. indukt. Abstamm. u. Vererb. **32**: 108–232.

MORGAN, L. V., 1922 Non-criss-cross inheritance in *Drosophila melanogaster*. Biol. Bull. **42**: 267–274.

MORGAN, T. H., 1915 The role of environment in the realization of sex-linked Mendelian character in Drosophila. Amer. Nat. **49**: 385–429.

MORGAN, T. H., and BRIDGES, C. B., 1916 Sex-linked inheritance in Drosophila. Carnegie Inst. Washington Publ. **237**: 87 pp.

MORGAN, T. H., BRIDGES, C. B., and STURTEVANT, A. H., 1925 The genetics of Drosophila. Bibliographia Genetica, vol. 2, 262 pp. The Hague: Martinus Nijhoff.

MORGAN, T. H., STURTEVANT, A. H., and BRIDGES, C. B., 1923–25 The constitution of the germ-material in relation to heredity. Year Book Carnegie Inst. Washington **22**: 283–287, **23**: 231–236 and **24**: 286–288.

NORTHROP, J. H., 1917 The effect of prolongation of the period of growth on the total duration of life. Jour. Biol. Chem. **32**: 123–126.

1917 The role of yeast in the nutrition of an insect (Drosophila). Jour. Biol. Chem. **30**: 181–187.

PEARL, R., PARKER, S. L., and GONZALEZ, B. M., 1923 Experimental studies on the duration of life. VII. The Mendelian inheritance of duration of life in crosses of wild type and quintuple stocks of *Drosophila melanogaster*. Amer. Nat. **57**: 153–192.

PICTET, A., 1902 L'influence des changements de nourriture sur les chenilles et sur la formation du sexe de leurs papillons. Compte Rendu Soc. Physique et Histoire Naturelle de Genève **19**: 66–69.

PLOUGH, H. H., 1919 The effect of temperature on crossing over. Jour. Exp. Zool. **24**: 147–209.

ROBERTS, E., 1918 Fluctuations in a recessive Mendelian character and selection. Jour. Exp. Zool. **27**: 157–192.

SEYSTER, E. W., 1919 Eye facet number as influenced by temperature in the bar-eyed mutant of *Drosophila melanogaster* (ampelophila). Biol. Bull. **37**: 168–182.

STURTEVANT, A. H., 1920 Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *D. melanogaster*. Genetics **6**: 179–207.

CYTOLOGICAL AND MORPHOLOGICAL INVESTIGATIONS ON GYNODIMORPHIC AND NORMAL FORMS OF *RANUNCULUS ACRIS* L.

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INTRODUCTION

The present studies were undertaken to determine whether certain morphological deviations from the typical vegetative and floral characteristics of *Ranunculus acris* L. are accompanied by variations from type in the nucleus. Work has been in progress for a number of years, and has involved many races of *Ranunculus acris* L., collected in Russia, Czechoslovakia and America, and cultured at CHARLES' UNIVERSITY, Prague, COLUMBIA UNIVERSITY and the UNIVERSITY of MINNESOTA.

Specifically, the investigation has involved:

(a) A study of the superficial morphological features of aberrant individuals and races of *R. acris* and the assembling of materials for genetic and cytological investigation.

(b) A cytological investigation of the behavior of the nuclei during somatic and reduction divisions with special reference to the problem of the individuality of the chromosomes.

(c) A study of the possible correlation between chromosome number and chromosome formulae with superficial morphological characteristics in aberrant races.

It is a pleasure to acknowledge my indebtedness for many valuable suggestions to Doctor B. NEMEC under whose direction this problem was begun. I wish also to express my obligations to Doctor R. A. HARPER and Doctor C. E. ALLEN for helpful criticism, to academician Doctor S. G. NAWASCHIN for the preparations of satellites, to Doctor C. O. ROSENDAHL in whose laboratories the work was completed for advice and criticism, and to Doctor J. ARTHUR HARRIS for help in the preparation of this paper.

EARLIER OBSERVATIONS ON GYNODIMORPHISM IN *Ranunculus acris* L.

Gynodimorphism has been described several times in different representatives of the family Ranunculaceae. Plants of *R. acris* L. with small flowers and abortive anthers were first reported growing with normal plants on the meadows at Politz, Mettau, by VELONOVSKY (1900). He says:

"Es sitzen überall auf den Blütenasten auffallend kleine Blüten (2–3 Mal kleiner als bei der Normalpflanze) welche kleine, mehr keilformige Petalen besitzen und auf dem Blütenboden nur Fruchtknoten aber keine Antheren tragen. Diese sind ganz unansehnlich unter den Fruchtknoten als Höcker versteckt. Die Fruchtknoten gelangen regelmässig zur vollstandigen Entwicklung und reifen sogar zu Früchten."

MAGNUS (1900), discussing the phenomenon described by VELONOVSKY, pointed out the importance of the gynodimorphic race:

"Auch die Bestaubungseinrichtungen der einzelnen Arten sind nichts starres, sondern etwas Gewordenes und Werdendes."

Near Grünberg, Germany, SCHMIDT (1911) found *R. acris* L. with small flowers, from ½ to ¼ of the size of the normal, with anthers fully aborted, petals and stamens often modified, increased in number, and even sometimes forming absolutely double flowers.

TURRILL (1919) mentioned the occurrence of similar flowers in *R. auricomus*, *R. acris* and *R. bulbosus*:

"Their stamens were very much reduced in size and produced little or no pollen. Similar unisexual states of both *Ranunculus bulbosus* and *R. acris* have been found on the lawns at Kew."

Gynodimorphic flowers of *Ranunculus polyanthemus* were observed by DOMIN (1923). Similar flowers in other Ranunculaceae are mentioned by PENZIG (1922).

The somewhat frequent occurrence of these aberrant types in Ranunculus and its relatives suggested the desirability of a cytological investigation with a view to determining whether nuclear, and particularly chromosomatic, phenomena are associated with the externally visible morphological characteristics.

FIGURES—1 to 25.

MATERIALS OF PRESENT INVESTIGATION

Gynodimorphic plants of *Ranunculus acris* L. were found by the writer at Czarskoe Selo, near Petrograd, Russia, in practically every place where one would expect to find the normal plants of *Ranunculus acris*. The pure gynodimorphic plants were rather rare as compared with the normal plants. Intermediate stages between the normal plants and the gynodimorphic were more abundant.

In figure 1 a typical gynodimorphic flower is compared with the normal (figure 2). The flowers of the gynodimorphic plant were small, their petals averaging from 3 to 4 mm in length, as compared with petals 10–11 mm in length in the normal plants. The stamens were abortive and were reduced to small staminodia. The pigment of the petals in the gynodimorphic flowers was very pale, as contrasted with the bright yellow pigment of the normal plants. Such vegetative characters as shape and size of leaves, the distribution of the hairs, and other characters were also conspicuously affected and involved in the variations. Intermediate forms, which in the degree of reduction of the anthers, and in size of the flowers approached either the extreme gynodimorphic or the normal form, were present.

The range of variations of stamens and pollen grains which have been observed in these forms is represented in figures 3 to 12. An anther and pollen grains of a normal *R. acris* are illustrated in figure 3. Figures 4 and 5 represent forms with slightly modified anthers and with apparently normal pollen. Forms in which the filaments are long, but the pollen sacs are rather strongly modified and provided with pollen grains irregular in size and shape are shown in figures 6 and 7. In the forms represented in figures 8, and 9 the anthers are further reduced and approach more nearly to the true staminodia. The pollen grains, if present, are extremely irregular. A form in which the pollen sacs, though present, are empty is shown in figure 9, (right). Some typical staminodia are shown in figures 11. The last stage of the reduction of the anthers to very small staminodia of a typical gynodimorphic plant is represented in figure 12.

A comparison of the figures of the forms discussed shows that there probably is a correlation between the degree of reduction of the anthers and the development of the hairs on the filaments of the stamens. The size and shape of the pollen grains in the intermediate forms are highly variable. The form shown in figure 6 has a great number of very large and irregular pollen grains. The form shown in figure 7 has on the contrary a great proportion of small-sized irregular pollen grains. In the form

shown in figure 8 there are still more irregularities in the shape and size of the pollen and a still greater proportion of shriveled grains.

In the spring of 1922 the writer collected gynodimorphic and normal plants of *R. acris* from the environs of Petrograd, Russia. In the fall of 1922 the seed material from these two forms was sown in the botanical garden of CHARLES' UNIVERSITY, Prague. In the summer months of 1923 the cultures flowered abundantly. The progeny of the gynodimorphic plants showed very pronounced variations of the vegetative characters and of the floral structures. These forms have received especial attention from the cytological standpoint.

MITOSES AND MEIOSES IN THE NORMAL AND GYNODIMORPHIC *R. acris*.

In the somatic mitoses (SOROKIN 1924) the normal plants were found to have 12 chromosomes, whereas the gynodimorphic had 18. Gynodimorphic plants with other chromosome numbers were also found later In both forms satellites were present in two chromosomes. In the normal plant one small satellite was connected with one chromosome, while the other chromosome had a satellite of slightly larger size. In the gynodimorphic plant three satellites were present, two of them connected with one chromosome, and one with another chromosome. The behavior of the nucleolus, which underwent regular divisions before the division of the nucleus in both normal and gynodimorphic forms, has been discussed (SOROKIN 1924).

The meiotic phase of one of the normal races of *R. acris* is described in detail in a separate paper, SOROKIN (1926). Here an inquiry into the individuality and morphology of the chromosomes will be considered.

In the cytological work several fixing reagents were used. For different purposes the solutions proved to be of unequal value. For root tips, the best preparations were obtained from the material fixed in chrom-formalin according to the new formula given by Professor B. NEMEC, which he recommends for fixing mitochondria,[1] and which was excellent for the chromosomes also. For studies of the reduction divisions, the best material was obtained from Flemming's medium. While Flemming's strong solution produced very pronounced plasmolysis, fairly good results were obtained from the picric-acetic-sulphuric acid killing solution. It is interesting to point out that the results of the fixation of the same plant with the same solution varied greatly and seemed to depend upon the

[1] 100 cm³ of 1 percent CrO_3 and 8 cm³ of formaldehyde were allowed to act on the material for 6–8 hours, after which the material was subjected to the action of a new prepared portion of the same solution for 16–18 hours.

time of day and upon the temperature of the air. The best results were always obtained from the material fixed during warm weather in the morning. For the study of the somatic mitoses sections were cut 7.5μ thick, and for the reduction divisions from 10μ to 15μ, and were stained with Heidenhain's iron alum haematoxylin.

The figures of the accompanying illustrations are all drawn with camera lucida .to corresponding scales. Namely: mitoses and meioses—Zeiss Apochr. 3 mm Ap. 1.30, comp. oc. 12; anthers, staminodia and hairs on the calyx of the progeny of a gynodimorphic plant crossed with normal *R. acris*—Leitz, 3.3; pollen grains 3.7, all these figures are diminished four times. Flowers—natural size. Range of variations of stamen and pollen grains in forms found growing wild—enlarged about 30 times.

EARLIER WORKS ON THE INDIVIDUALITY OF THE CHROMOSOMES

Studies on the individuality of the chromosomes have recently acquired great importance. The relative size of the chromosomes, peculiar shape, and especially the presence of satellites (S. NAWASCHIN, 1912) and constrictions (SAKAMURA 1915, 1920) furnish criteria for distinguishing the members of a given complement, or using the terminology of S. NAWASCHIN, they characterize the *idiogram* or the specific arrangement of the diploid nuclear plates.

S. NAWASCHIN (1912) discovered the satellites of the chromosomes in *Galtonia candicans*. The following data are given by him (1912, 1915) concerning the nature of these peculiar structures. The satellites are much smaller than the chromosomes. In the metaphase of the somatic mitoses they are attached by means of delicate threads to a certain chromosome pair. They appear already during the prophases, where they were observed on the surface of the nucleoli. During the maturation divisions the satellites fuse with their chromosomes, reappearing again in the pollen grains during the divisions of the primary nucleus.

The writer was very fortunate to receive from Prof. S. G. NAWASCHIN excellent preparations of *Galtonia candicans* showing satellites of the somatic chromosomes in prophases and metaphases. Slides made from the material killed with sublimate osmic and stained with Delafield and eosin, showed in the prophases the dark purple stained satellites situated on the opposite sides of the nucleolus, which was stained pink with this combination of stains.

In the diploid nuclei of *Drimiopsis maculata* BARANOV (1926) found four large and 12–16 small satellites. From the beginning of the prophases till the moment of the formation of the chromosomes, the satellites are

found on the surface of the nucleoli. Later on they are picked up from this position by the delicate threads extending from the ends of the chromosomes, and appear to be connected with the chromosomes during the metaphases and anaphases. In the heterotypic division, the satellites were observed only in leptonema and synizisis stages. In the same paper BARANOV refers to the slides found now at the TIMIRIAZEFF'S INSTITUT, Moskaw and to the paper of S. G. NAWASCHIN given in Vienna in 1913, in which the arrangement of the satellites on the nucleoli in the prophases is clearly demonstrated.

In a recent paper M. NAWASCHIN (1925b) describes the satellites in diploid and triploid *Crepis capillaris*, where they are found on the surface of the nucleoli during the prophases. Two satellites are found on the nucleoli of the diploid race, and three satellites are recorded on the nucleoli of the triploid individuals.

The number of plants in which the satellites are found is steadily increasing. The important literature on the subject is outlined in the book by TISCHLER (1921–1922), and in the articles by TAYLOR (1924, 1925a, 1925b, 1925c), NAWASCHIN (1925a) BARANOV (1926). In addition to the recorded literature a recent paper by MEYER (1925) should be mentioned. He confirmed the previous observation of the satellites in somatic mitoses of *Leontodon autumnaeis*. The satellites were found also in the polyploid nuclei of the tapetal cells, but absent in the chromosomes during the reduction division. NAWASCHIN (1925a) published the first results of his intensive studies on the chromosomes of fourteen species of Crepis. In all the investigated forms two distinct types of the chromosomes were found. The first type was represented by the two-armed, slightly constricted chromosomes, the arms being usually of unequal length. The second type included rod-shaped chromosomes with small proximal satellites. Miss EMME (1925) studied the idiograms of a great number of varieties of wheat. She found that endemical forms from Asia mostly do not have satellites. The endemical forms from Africa have one satellite, and belong to the asymmetrical race. The wheat varieties which do not belong to any of the endemical groups have different karyotypes, partly symmetrical with no, or with two satellites, and partly asymmetrical with one satellite. In spite of the fact that more than one hundred plants with satellites are recorded, their nature is often misunderstood and confused with some of the other typical characters of the chromosomes. The conception of the satellite of DARLINGTON (1926) as being "the result of a local discontinuity which occurs in different chromosomes at different positions—bisecting the chromosome or cutting off a minute

element, which will form, in the contracted state, a satellite" is entirely wrong. The satellites have a different origin and always represent hereditary morphological characteristics of the chromosomes. As to the phenomenon observed by DARLINGTON, it must have been the type of temporary constrictions which sometimes occur in the chromosomes.

TAYLOR (1925c, 1926) recognized three notable features of the chromosomes, namely constrictions at the zone of fiber attachment, constrictions unrelated to the fiber attachment, and satellites. In regard to the second type of morphological characters of the chromosomes TAYLOR (1926) thinks that there does not seem to be distinct demarcations between this kind of phenomena and the original satellites. Somewhat similar conclusions could be made from the paper of DELAUNAY (1925) on Ornithogalum. In different species of this plant he found satellites of unequal length. The longest satellites being in *O. umbellatum* and *O. Narbonense*, the shortest in *O. oligophyllum*. This conception of the satellites differs from the original description of NAWASCHIN and his pupils. In order to avoid misunderstanding it would be desirable to restrict the word "satellite" to the phenomena which approach the original satellites in *Galtonia candicans*.

Besides the two permanent types of constrictions, one related to the fiber attachment, the other independent, there are sometimes found in the chromosomes temporary constrictions, which are not distinct hereditary characters, and which may appear and disappear at certain periods of the development of the chromosomes. The constrictions of the chromosomes in prophases of the somatic and heterotypic divisions, and especially the constrictions related to the interchange of the segments of a certain pair of the chromosomes belong to this temporary type. These constrictions occur in *Ranunculus acris* (SOROKIN 1926) shortly before diakinesis. The constrictions of the chromosomes with the subsequent fracture (BELLING 1925) should also be considered in the class of temporary constrictions.

THE INDIVIDUALITY OF THE CHROMOSOMES IN *R. acris*

In *R. acris* the individuality of the chromosomes is readily demonstrable. The somatic chromosomes differ in size, shape, presence of constrictions, and satellites.

The formula for the somatic chromosomes of the normal race of *R. acris* from Czarskoe Selo, Petrograd, is $2A+2B+2c+2d''+2e+2f=12$ chromosomes. The chromosomes in this formula are arranged first of all according to differences in size. The two A-chromosomes are very large.

They are, therefore, indicated by capital letters. The B-chromosomes are medium large, while the c-, d- and e- chromosomes are medium and the f-chromosomes are very small. The second distinguishing character is shape. The A-chromosomes are V-shaped, with both arms long, of equal size and with median fiber-attachment. These chromosomes are easily recognized in different stages of mitosis, even under unfavorable conditions. The B-chromosomes are hook-shaped with one very long arm, which is sometimes even a little longer than the arms of the A-chromosomes, and with another arm one-third as long as the first. The fiber attachment is subterminal. A very marked constriction is found between the two arms of the B-chromosomes. The c-chromosomes are of regular small v-shape. The d-chromosomes are hook-shaped, with one arm one-fourth as long as the other. Either both of the d-chromosomes are provided with the satellites and constitute the symmetric race, according to the terminology of S. NAWASCHIN (1915) or only one of the d-chromosomes has a satellite and can form an asymmetric race. Accordingly the presence of one or two apostrophics in the formula after the letter d will indicate the condition. The e-chromosomes are either of rod or hook shape and without any particular properties in the somatic mitoses. The f-chromosomes are very small rods, and are always found to be in the center of the metaphasic figures.

The shape and size of the individual chromosomes in the meiotic phase of the normal *R. acris* from Czarskoe Selo are represented in figures 13, 14. The A-chromosomes form a large cross in diakinesis (figure 14A). The B-chromosomes (figure 14B) form two curves which are crossed once or twice, the c-chromosomes form a small cross (figure 14C) the d-chromosomes are connected on one side (figure 14D), the e-chromosomes, (figure 14E) remain for a very long time connected with the nucleolus, and the f-chromosomes (figure 14F) of very small size, form two very small rods.

In the gynodimorphic plants the conditions as to the individuality of the chromosomes are extremely complicated. The original gynodimorphic plant which is represented in figures 1 and 12 and which was used as a parent plant for the subsequent generations had 18 chromosomes. Because of limited material these chromosomes were not identified exactly as to their individuality, but it did appear that the chromosomes of this plant, though present in the number of 18 or in a trivalent number, did not represent a triploid race, because they could not be arranged in six trivalent sets. Chromosomes A and B could never be found in triple numbers. As will be shown later purely gynodimorphic plants with varying numbers of chromosomes have been found among the offspring

of the original gynodimorphic plant with 18 chromosomes crossed with the normal plants. The figures in table 1 which gives the somatic formulae show that these numbers were 13, 15 and 18. It must be pointed out that the original gynodimorphic plant which was used in the experiment as the parent plant was extremely tall (about 120 cm) while the gynodimorphic plants with 13 and 15 chromosomes did not exceed 50 cm.

DESCRIPTION OF THE PROGENY OF A GYNODIMORPHIC PLANT CROSSED WITH NORMAL *R. acris*

The seeds from a gynodimorphic plant of *R. acris* grown isolated in the experiment garden of the AGRICULTURAL ACADEMY in Czarskoe Selo, near Petrograd, Russia, and fertilized by the pollen of normal plant of the same species, were sown in Prague, Czechoslovakia. The progeny was extremely variable in morphological characters. The size and shape and the degree of lobing of the leaves from entire to finely dissected, the distribution of the hairs on the leaves, varied considerably in all these forms. Gigantism and dwarfness were quite usual phenomena. Some plants were remarkably tall, others were of the type of the nanella mutations. But most conspicuous were variations in the floral structures. About one-quarter of the offspring were typical gynodimorphic. Most of the plants, however, showed intermediate stages between the purely gynodimorphic and the normal types. Only a very limited number of wholly normal plants could be found among the offspring.

Among other remarkable variations in the floral structures were reduction of the size of the sepals and petals and increase in the number of these organs. Some of the plants did not flower at all during the first season, so it was impossible to give accurate percentage of the different types of flowers.

The number of the somatic chromosomes of the different types of forms and the reduction division of the forms in which the anthers were not abortive were studied. The following description will illustrate the relations between the morphological characters and the cytological structures.

(a) *Mutations*. Among the progeny of the above plant, two forms were distinguished as mutations. They showed profound modifications of the structure of the flowers and were characterized as independent, absolutely different races, without any intermediate transition forms between them, and the other forms. They were called "Open buds" and "Asepalis." Both these plants began to flower in my cultures earlier than any of the others, and later on they produced abundant and apparently normal seeds.

The "Open bud" form showed complete reduction of sepals, reduction of petals to small scales, reduction of the anthers to staminodia and comparatively normal pistils, which were situated on a rather elongated receptacle. The whole aspect of the flower, of which figure 15 gives an illustration, was very unusual. The buds of this form were completely open and the young pistils were exposed from a very early stage. Longitudinal section of a bud, figure 16, gives an illustration of the parts of the flower of an "Open bud" mutation. The exact number and the individuality of the somatic chromosomes of this form has not been determined for want of material. There is, however, some evidence that the number is low, approximately 12–14. Some irregularities in the behavior of the chromosomes in this form were found which have never been seen in any of the other investigated forms. These irregularities were observed in the late anaphases and early telophases of the somatic mitoses. The anaphasic chromosomes pass to the poles rather separated from each other (figure 17). After the poles have been reached they become more closely associated (figure 18). During this time it is possible to observe on the spindle two distinct groups of chromatin structures which form two very regularly arranged rows of small rods. These groups extend toward the periphery of the cell, become transformed into irregular masses, and by the telophase (figure 19) gradually disappear. This peculiarity was observed in somatic mitoses of the different parts of the plant, and roughly resemble the bridges of the lagging chromosomes, which have been described in the meiotic phases in the *Papaver* hybrids by YASUI (1921) and LJUNGDAHL (1922, 1924).

The "Asepalis form" was characterized by the absence of the sepals as the name indicates. The early buds were covered directly by the bright yellow petals. In other respects these mutations were quite normal. The chromosome number of this mutation was the same as in the normal form with the difference (a) that the outlines of the individual chromosomes were not so distinct, and (b) the peculiar shape and size were not so pronounced.

The seeds collected from the two mutating forms were sown and some of the offspring plants have already flowered. The above described characters were transmitted to part of them. The cytological investigation of these forms is not yet completed.

(b) *Forms with different degrees of reduction of the anthers.* As already stated the majority of the progeny of one gynodimorphic×normal plant showed a somewhat intermediate condition of the degree of the reduction of the anthers and of some other characters of the floral structure. For

convenience all the forms investigated have been divided into six classes, according to the degree of the reduction of the anthers. From every class are described one or several representatives in which the behavior of the chromosomes was found to be different from that of the other classes, or from the forms of the same class.

The first class (represented in figures 20–25) includes forms with complete reduction of the pollen sacs, namely with purely staminodial flowers in which the staminodia are very small (from 3 to 4 mm). In the second class forms are included in which pollen sacs are absolutely reduced but the staminodia are somewhat enlarged at the tips (figures 26–30). The forms of the third class show some rudiment of the pollen sacs (figures 31–34). Increase in the size of the pollen sacs and of the amount of pollen in these sacs is the outstanding characteristics of the fourth class (figures 35–47). The fifth class includes the forms in which all four pollen sacs are developed, but they still differ in shape and size from the ordinary pollen sacs (figures 48–52). The sixth class includes the forms which have normal pollen sacs.

In addition to the other characteristics of the floral structure which were variable in the forms investigated should be mentioned the degree of development of the hairs on the sepals, a feature which is important from the taxonomic point of view.

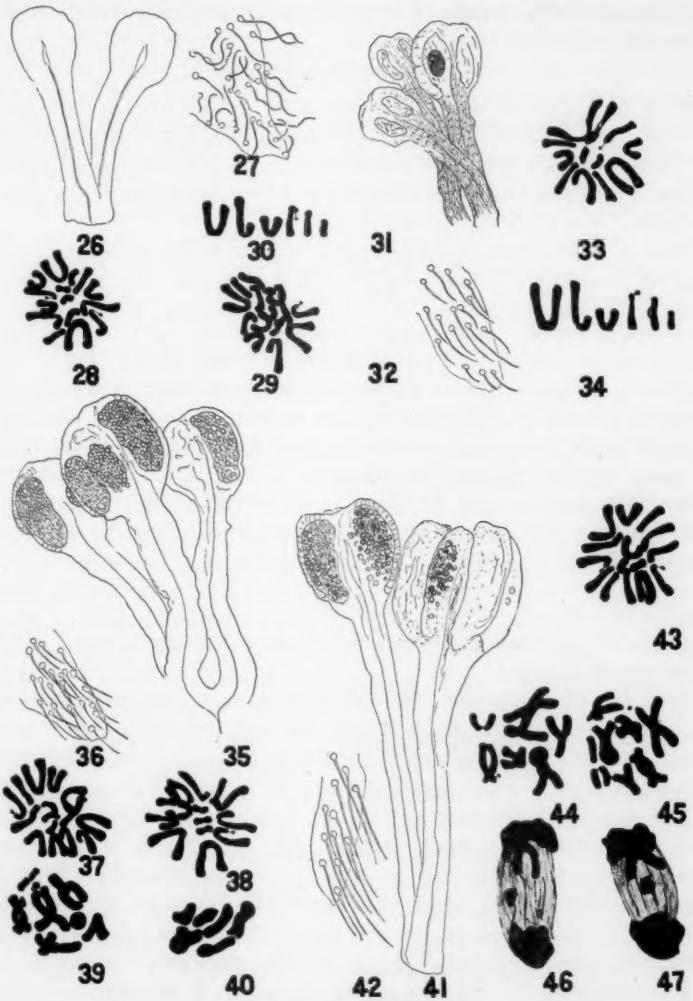
Class I

Form 1. (Figures 20–23). Petals small of unequal size, varying from 5 to 5.5 mm in length; staminodia very small, without any enlargement of the tips, and without any rudiments of the pollen sacs, beset at the base with a few rather short, straight hairs which are found also on the calyx (figure 21).

Chromosomes 13. It was possible to distinguish the individuality of the chromosomes in the metaphases (figure 22) and to establish the somatic formula (figure 23) $2A+2B+2c+2d''+3e+2f=13$. The two d-chromosomes were provided with satellites of slightly different size. The additional chromosomes were of the rod-type and were of the shape and size approaching the e-chromosomes. In other respects the somatic mitoses of this form did not differ from that of the normal.

Form 2. Plants morphologically absolutely identical with the original gynodimorphic parent were found in this progeny to have 18 somatic chromosomes. As in the mother plant, the chromosomes in this form did not form a triploid complement. The 12 chromosomes could be distinguished by their size and shape, and were identical with the 12

chromosomes of the diploid forms. The 6 chromosomes were of medium size and approached in the shape the chromosomes of the e-type. The exact somatic formula, however, could not be established. It was nevertheless clearly evident that the large chromosomes of the A and B types were present in diploid numbers.



FIGURES—26 to 47.

Form 3. (Figure 24). Petals extremely small (from 3 to 3.5 mm in length; staminodia very small.

This form had the same number of chromosomes and the same somatic formula as form 1.

Form 4. (Figure 25). A form with rather pronounced tendency towards hairiness of the staminodia. Chromosomes 15, somatic formula: 2A+2B +3c+2d''+3e+3f.

Class II

Form 5. (Figures 26–30). Flowers small; petals from 4 to 4.5 mm; staminodia were somewhat larger than in preceding forms, a little enlarged at the tips, hairs wanting on staminodia; calyx beset with crinkled hairs (figure 27). Chromosomes in the somatic metaphases (figures 28, 29), 15. Formula: 2A+2B+3c+2d''+3e+3f.

Form 6. In contrast to the preceding, this form had rather large petals ranging from 8 to 8.5 mm in length; the staminodia were identical with those of the preceding form. The chromosomes were not determined.

Class III

Form 7. (Figures 31–34). Flowers extremely small; petals from 3.5 to 4 mm in length. Calyx beset with short hairs (figure 32). Staminodia without hairs; pollen sacs somewhat developed, but the pollen lacking in some, mostly imperfect in others.

Somatic chromosomes, 12, formula as in the normal form, 2A+2B +2c+2d'+2e+2f (figures 33, 34). One satellite of the d-chromosomes was established but there was no certain evidence of the other.

Class IV

The forms included in this class all developed abundant but somewhat irregular pollen grains.

Form 8. (Figures 35–40). Flowers of medium size; sepals covered with very abundant straight hairs (figure 36), petals from 5 to 5.5 mm in length. In certain reduced anthers all four pollen sacs developed, in others only three or even two present, and of rather irregular shape (figure 35).

The diploid chromosome number, as determined in the root tips and in the cells of the developing ovary, 15. The arrangement of the chromosomes in two metaphasic plates is shown in text (figures 37, 38). The somatic mitoses did not show any irregularities, and the division of the nucleolus during the early somatic prophases, as has been described as typical for other forms of *R. acris*, was also present.

The reduction division was studied in detail in this form. Only the principal phases, which are directly connected with the distribution of the

chromosomes in the gametes will be discussed here. The arrangement of the chromosomes in bivalents and trivalents during the diakinesis occurs from the very beginning of this stage, as contrasted with the facts which were described in the similar stages in triploid *Rubus* and *Crataegi* by LONGLEY (1924a, 1924b).

The diakinesis stage represented in figure 39 shows very distinctly three bivalent and three trivalent sets. The two large chromosomes form the usual large cross, the two B-chromosomes are still distinguished by double crossed shape while the small cross is formed here by the union end-to-end of a third chromosome together with the two bivalents. The d-chromosomes form a bivalent, while the e-chromosomes and the f-chromosomes are quite distinctly associated into trivalents. The e-chromosomes form a ring with a rod connected somewhere to the ring, and the f-chromosomes never cross, and are seldom connected, but simply lie side by side. During late metaphase six sets are present, but it is impossible to distinguish which of them are the bivalents and which are the trivalents, (figure 40). During the anaphase the lagging chromosomes show that the distribution of the bivalents and trivalents is random. The resulting pollen grains receive different chromosome numbers and are of different size and shape. In the somatic mitoses the same peculiar individuality of the chromosomes was established. In most of the preparations studied it was possible to distinguish the two homologous chromosomes of the types A, B and d. The remaining chromosomes of a typical diploid complement were always found to be in the number of three's. Somatic formula: 2A+2B +3c+2d''+3e+3f.

Form 9. (Figures 41–47). Flowers rather large, petals from 7 to 7.5 mm in length; each anther containing four pollen sacs; filaments very long, without hairs; pollen very irregular in size and shape; sepals with very long straight hairs (figure 42). The stamen to the right in figure 4 has shed the pollen, while in the one to the left the pollen grains are still present. Figure 43 represents a metaphase of the somatic mitoses. The number of the bivalents in the diakinesis of heterotype division was determined to be 6: the remaining chromosomes formed a trivalent. Figures 44, 45 represent diakinesis of the reduction division in the pollen mother cells. The individuality of the chromosomes is clearly distinguished. The A chromosomes always form a very distinct large cross during this stage. The B-chromosomes are distinguished by the typical double cross shape. The c-chromosomes form a small cross, the d-chromosomes are connected at one end and crossed at the other, or they may be open at the other end. The f-chromosomes never cross, and are very

rarely connected with each other, forming more or less closely approximated small rods. The e-chromosomes are as usual connected with the nucleolus for a very long time, and associated with them it is possible to observe a third chromosome of the same size (figure 45). In some of the figures, however, the third chromosome is connected with one extra pair (figure 44) which is approximately of the size of the e-chromosomes also, but which is separated from the nucleolus. This third chromosome can lie very close to the extra pair, or it can be connected with it either in the form of a ring with an attached rod, or in the form of a Y-shaped arrangement. Both forms of connection of the trivalents are described for triploid Daturas by BELLING and BLAKESLEE (1923).

During the metaphases the chromosomes shorten but still retain their peculiar characteristics. The anaphases shown in figures 46 and 47 represent the irregularities which have been found in this stage. Lagging chromosomes are present on the spindle.

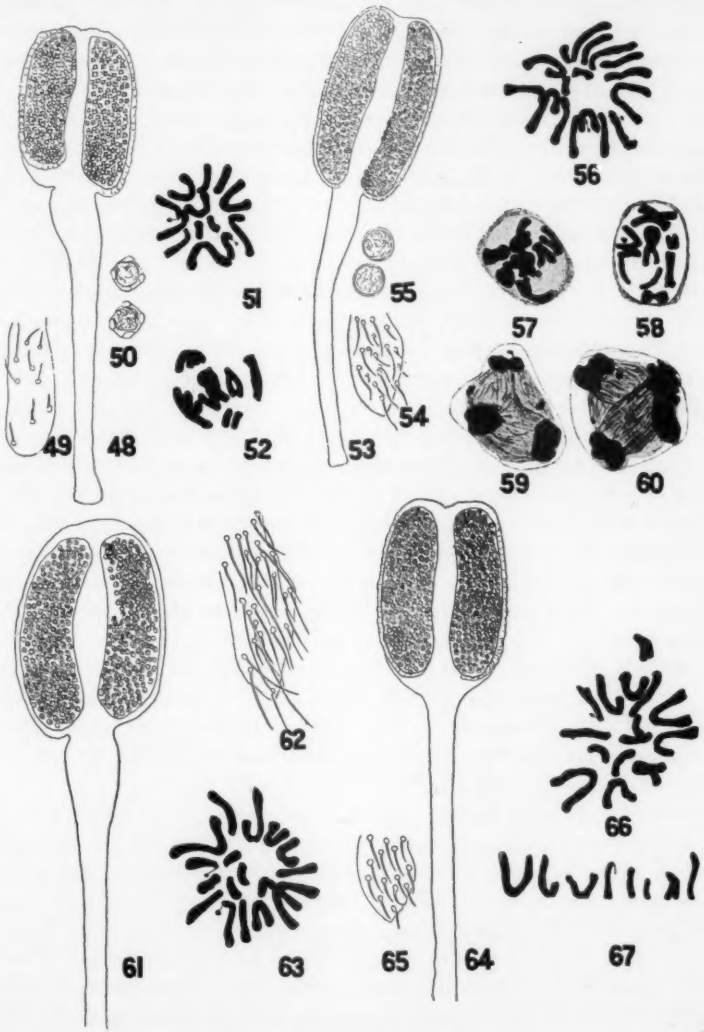
The degeneration of the spindle shows some irregularities. After the chromosomes have reached the poles, the fibers of the spindle thicken and become very pronounced. It is easy to observe that it is made up of small granules, which gradually disappear. The homotype division proceeds comparatively regularly and the resulting pollen grains receive different chromosome numbers as a result of the irregularities during the heterotype division. The large amount of shriveled and irregular shaped pollen grains is an additional illustration of the random distribution of the chromosomes in the gametes.

Taking into consideration the behavior of the chromosomes during the heterotype division, the somatic formula for this form will be: $2A+2B+2c+2d''+3e+2e_1+2f$.

Class V

Form 10. (Figures 48–52). Flowers of normal size, sepals covered with a few short hairs (figure 49); anthers developed almost as in the normal form; pollen sacs only a little shorter, slightly irregular; pollen abundant and apparently normal in function, shape of the grains though very constant, remarkably distinct from that of the normal form. The characteristic quadrangular pollen grains are shown in figure 50. The somatic number of the chromosomes is 13, formula: $2A+2B+2c+2d''+3e+2f$. Two satellites of unequal size are present on the two d-chromosomes of the somatic nuclei (figure 51). Studies of the reduction division showed that the chromosomes in the diakinesis and metaphase of the first mutation division formed regular 5 bivalents and 1 trivalent. Figure 52 gives illustration of this stage.

Form 11. (Figure 53–60). Flowers of normal size; sepals covered with very short but rather abundant hairs, (figure 54) anthers developed almost as in the normal form. The somatic number of chromosomes, 16; the



FIGURES—48 to 67.

outlines of the individual chromosomes sometimes not so regular as in all the preceding forms.

Two satellites were present on the two d-chromosomes. In figures 57–60 are represented the most significant stages in the development of the pollen grains. The figures 57 and 58 represent diakinesis of the first maturation division. There are present 8 very distinct pairs of chromosomes. The A-chromosomes form the large cross, which in the side view can be seen as if one chromosome is overlapping the other. The two second chromosomes of the large size are the B-chromosomes. They are clearly distinguished by their size and peculiar constrictions. The c-chromosomes form the regular small cross in both figures, the d-chromosomes are accompanied by a small body, which is apparently of the type of temporary constrictions. In figure 57 it seems as if this body is connected with the d-chromosomes, whereas in the figure 58 this is not so clear. The e-chromosomes remain connected with the nucleolus for a very long time. Two additional pairs were approximately of the same size and shape as the e-chromosomes. In the figure 58 the chromosomes of one of these additional pairs to the regular complement seems to fail to be closely associated at any rate during the diakinesis. The small f-chromosomes constitute the eighth pair of the complement. No good preparations were obtained from the other stages of the meiotic phase except the telophases of the homotype division. As far as it was possible to judge concerning the distribution of the chromosomes in the gametes from these stages it seems that it was comparatively regular. Lagging chromosomes were not present, the black bodies found in the figures were always the extruded bodies which have been described by several authors in the telephases of the meiotic divisions. The four pollen grains which resulted from the divisions of the pollen mother cells were all of the same size and shape. As a result of the comparison of the behavior of the chromosomes during mitoses and meioses the somatic formula was established as: $2A+2B+2c+2d''+2e+2e_1+2e_2+2f$.

Form 12. (Figures 61–68). Flowers very large, even larger than in the normal form; petals and sepals increased in number from 6–12; sepals covered with very long and very abundant straight hairs (figure 62); petals from 11 to 12 mm in length. The somatic chromosome number is 17 (figure 63) two of the chromosomes are provided with satellites of unequal size. Material for definite conclusions as to the reduction division of this form has not been obtained. From the studies of the somatic divisions, however, it was possible to determine approximately the number of chromosomes and the formula as: $2A+2B+3c+2d''+4e+4f$.

Form 13. (Figures 64–67). Flowers of normal size; anthers apparently normally developed. In the somatic complement some irregularities were found. The form had 15 chromosomes (figure 66); all were very well distinguished by their peculiar shape, size and some individual characteristics. The A-chromosomes were arranged opposite each other, the same position was found for the c-chromosomes, and the e- and the f-chromosomes formed very regular pairs in the metaphasic plate. In the d-chromosomes satellites of different size were present. The three additional chromosomes could not be arranged under any of the categories; two of them apparently formed a good pair and were of the c-chromosome type, the third was very long and rod-shaped.

The following table will summarize the different number of chromosomes which were found in the representatives of the different classes, and sometimes in the representatives of the same class.

TABLE 1

Somatic formulae of the progeny of the gynodimorphic × normal R. acris.

CLASSES AND NUMBER OF THE INDIVIDUALS	FORMULAE	CHROMOSOME NUMBERS
Class I.		
Form 1.	2A+2B+2c+2d″+3e+2f	13
2.		18
3.	2A+2B+2c+2d″+3e+2f	13
4.	2A+2B+3c+2d″+3e+3f	15
Class II.		
Form 5.	2A+2B+3c+2d″+3e+3f	15
6.		
Class III.		
Form 7.	2A+2B+2c+2d″+2e+2f	12
Class IV.		
Form 8.	2A+2B+3c+2d″+3e+3f	15
9.	2A+2B+2c+2d″+3e+2e_1+2f	15
Class V.		
Form 10.	2A+2B+2c+2d″+3e+2f	13
11.	2A+2B+2c+2d″+2e+2e_1+2e_2+2f	16
12.	2A+2B+3c+2d″+4e+4f	17
Class VI.		
Form 13.	2A+2B+2c+2d″+2e+2f+3 (extra)	15

The above table affords the basis for the following conclusions:

(a) Different chromosome numbers are found in forms belonging to the same class. The gynodimorphism was found in *Ranunculus acris* in the

forms with different chromosome numbers. In form 13, where the morphological characters were absolutely identical with the normal form, the constitution of the nuclei was different.

(b) One and the same number of chromosomes, and even one and the same somatic fomula, were found in forms with every pronounced morphological differentiations. For instance, 15 chromosomes have been found in the forms of the first, second, fourth, and sixth classes. Further, the somatic formulae of form No. 4 of the first class, and that of form No. 8 of the fourth class were absolutely identical, although the morphological characters were different.

(c) There is no correlation between the number of chromosomes and the degree of reduction of the anthers. Both high and low chromosome numbers were found among the forms of the first class. The morphological differences between the forms of the first class having 18 chromosomes and that with 13 somatic chromosomes were very insignificant.

The somatic formulae which were established on the basis of the behavior of the individual chromosomes show that in most of the forms discussed the additional chromosomes formed trivalents during the heterotype division, and were distinguished as being triple mates of some chromosomes of the somatic complement. In one case, however, the diakinetic chromosomes formed instead of several bivalents and trivalents, eight distinct pairs, which later behaved as absolutely normal bivalents. In two cases on a row with some trivalents, there were present quite distinct and new additional bivalents. The presence of the trivalents in the meiotic nuclei is a very strong evidence for the theory of the origin of those forms from the crosses of the polyploid forms of *R. acris*. The strong affinity of the homologous chromosomes seems, however, in some forms to lose its efficiency and we find there instead of the trivalent new bivalents as in form 11 with 16 chromosomes.

As to the individual chromosomes which are involved in the variations, it is interesting to emphasize that the A and B chromosomes have never been found in triple numbers. Apparently the chromosomes of the d-type behave in a similar way. The remaining chromosomes are all found in triple numbers in different forms, but most commonly the e-chromosomes figure as the varying members. In almost all forms satellites have been found. In the majority of cases two satellites were present in every nucleus. In some of the forms not very definite evidence was found concerning the second satellite. Possibly it was absent and we have an example of the asymmetric races of S. NAWASCHIN (1912).

Different results were obtained by BELLING and BLAKESLEE (1922) in investigations of the progeny of the triploid Daturas pollinated by diploid. The plants with one extra chromosome showed that this extra chromosome could be of the extra large, large, medium, small or extra small types.

DISCUSSION

It is evident from the above observations that gynodimorphism in *R. acris* is associated with somewhat unstable conditions of the nuclei, a condition to which BLAKESLEE (1921) gives the name of "unbalanced changes." A similar condition is typical for the hybrids between different species and for the progeny of the crosses between the polyploid races of the same species.

JEFFREY (1918) found a considerable proportion of imperfect pollen in *R. acris* and explained this condition as a consequence of previous hybridization. Theoretically both these conditions are quite likely to have taken place in the cases under discussion. But for *R. acris* from Russia direct evidence points rather strongly to the probability that the crosses between different polyploid races have played a more important role in the production of gynodimorphism than general polymorphism.

The first evidence against the interspecies hybrid nature of the forms under consideration is that so far as known hybridization between the species is not recorded to result in the degeneration of the stamens to the degree of staminodia. It is true that generally there is a tendency towards degeneration within the anthers in some of the hybrids as TISCHLER (1906) has described for Bryonia. LONGLEY (1924b) considers similar degeneration within the anthers of *Crataegus Smithii* as evidence for the hybrid nature of this species.

The second evidence against hybridization (having taken place in the modern times) as the cause of gynodimorphism in *R. acris* from Russia is that the only other species of Ranunculus found in flower at the same time with *R. acris* on the meadows and lawns at Czarskoe Selo, was *R. repens* L., a species with very pronounced morphological distinctions. Doctor DOMIN of Prague, a specialist of the *R. acris*×*repens* hybrids, examined my material and concluded that it does not resemble at all the known hybrids of the above mentioned species.

There is still another possible theoretical explanation of the origin of the gynodimorphic condition in *R. acris*, which could be the result of hybridization and subsequent segregation which may have taken place in the far past. The typical arrangement of the chromosomes during

meiosis in the hybrids between different species is that of the formation of the bi- and uni-valents, as described by ROSENBERG (1909). It is true that in a recent investigation LJUNGDAHL (1924) showed for Papaver hybrids the possibility of the associations of the chromosomes in pairs independent of their origin from different parents, or from the same parent. He calls this phenomenon autosyndese. Similar evidence was brought forward by HAASE-BESSEL (1921).

The study of the individuality of the chromosomes offers some possibilities for distinguishing the chromosomes and shows the occurrence of the peculiar triploid sets of certain chromosomes in the investigated progeny. All this furnishes good evidence for the theory (ERNST 1922; OVEREEM 1921, 1922; MOL 1921) which would explain the polymorphism in *R. acris* as a result of crosses between polyploid races and subsequent segregations. The gametes obtained as a result of similar crosses and fertilized by normal, or by modified ones are in their turn the cause for the further variations in subsequent generations. On the other hand they also can give rise to some forms, like the above described from 11, with 16 somatic chromosomes, in which the chromosomes will pair during the diakineses and metaphases, and the resulting gametes will have a constant chromosome number, different from that of the normal. Here we see an example in which the unbalanced conditions of the nuclei become balanced and results in the origin of a form with a new chromosome complement. It will be remembered, that the morphological characters of this form were slightly different from the normal form.

While carrying on the above investigations it seemed of interest to compare the plants of these cultures with the races of *R. acris* grown wild in America. With this purpose in view material of *R. acris* was collected from the meadows of the BOTANICAL GARDEN, Bronx Park, N. Y. Studies of the reduction division of this form is described separately (SOROKIN 1926). Here it is only necessary to note that this form has 14 chromosomes, which form 7 very distinct pairs during diakinesis, and that later, after the homotypic division is completed, the resulting pollen grains receive 7 chromosomes each. The divisions so far as studied appeared to be perfectly regular.

HOCQUETTE (1922) studying the number of chromosomes of *Ranunculus acris* L. subsp. *boreauanus* (Jord) Rouy et Fouc. from France found it to have 16 somatic chromosomes. For *Ranunculus repens* L. var. *typicus* Beck. which formerly was reported as having 24 chromosomes, HOCQUETTE (l.c.) gives 32 as the diploid number, and explains that the difference between the results of the investigators is due to the fact that

the different varieties and races of the same species may have different chromosome numbers. The same condition was found by the same author in another representative of the Ranunculaceae, namely in *Thalictrum minus*, in which there were previously reported 24 diploid chromosomes, whereas HOCQUETTE (l.c.) gives the number as 48.

LITARDIÈRE (1921) pointed out that in very closely related species, and even in the varieties of the same species, the number of chromosomes can be quite different. Further, either the varieties of a certain species with different chromosome numbers can differ morphologically, or on the contrary they can be absolutely identical as to their morphological characters. As examples of these statements LITARDIÈRE (1921, 1922) mentioned the following plants which have different cytological strains: *Salvinia natans*, *Pteris tremula*, *Podophyllum peltatum*, *Senecio vulgaris*.

It is quite probable that the races of *R. acris* the one from the BOTANICAL GARDEN, Bronx Park, N. Y., with 14 chromosomes in the somatic nuclei, and the other from Czarskoe Selo, Petrograd, with 12 diploid chromosomes, which are absolutely similar morphologically, represent an example of cytological races or strains in *R. acris*.

SUMMARY

1. Gynodimorphism in *Ranunculus acris* is associated with unstable condition of the plant characters and is an indication of the unbalanced types of changes which have taken place in the chromosome complement of the nuclei.

2. The progeny of a gynodimorphic plant by normal shows great variations in the morphological characters associated with the changes in the chromosome numbers. In the meioses in these forms irregularities are shown in the distribution of the chromosomes, and the resulting gametes receive different chromosome numbers. The union of the gametes with the modified chromosome numbers with the normal gametes or with the modified ones, is found to be responsible for the further changes of the chromosome constitution in the later generations and for the changes in the morphological characters.

3. The individuality of the chromosomes is found to be very distinct. The chromosomes in all investigated forms are distinguished according to their shape, size, presence of satellites and constrictions.

4. The presence of the trivalents in the progeny of the gynodimorphic plant by normal suggests that the unbalanced condition of the nuclei in these forms is a result of the irregular distribution of the chromosomes

during the meiotic phases, similar to that of the progenies of the polyploid forms crossed by normal or by other polyploids.

5. The occurrence of the different chromosome numbers in morphologically identical plants is an indication of the occurrence of cytological races in *R. acris* L.

6. Generally polymorphism in *Ranunculus acris* might be explained as a result of crosses between different polyploid forms and segregations taking place in the subsequent generations.

7. The unbalanced type of changes in the nuclei of the progeny of the polyploid forms by normal can be the beginning or initiation of the balanced types with different chromosome complements.

LITERATURE CITED

BARANOW, P., 1926 Cytologische und embryologische Untersuchungen an *Drimiopsis maculata*. Lindl. Zeitschr. Zellforsch. u. Mik. Anat. **3**: 131–148.

BELLING, J., 1925 The origin of chromosomal mutations in Uvularia. Jour. Genetics **15**: 245–266.

BELLING, J., and BLAKESLEE, A. F., 1922 The assortment of chromosomes in triploid Daturas. Amer. Nat. **56**: 339–346.

1923 The reduction division in haploid, diploid, triploid and tetraploid Daturas. Proc. Amer. Nat. Acad. Sci. **9**: 106–111.

BLAKESLEE, A. F., 1921 Types of mutations and their possible significance in evolution. Amer. Nat. **55**: 254–267.

DARLINGTON, C. D., 1926 Chromosome studies in the Scilleae. Jour. Genetics **16**: 237–251.

DELAUNAY, L., 1925 The S-chromosomes in Ornithogalum L. Science N.S. **62**: 15–16.

DOMIN, K., 1923 Příspěvky k morfologii a teratologii rostlin. Rozpravy České Akademie, **32**: Třida, II, nr. **18**: 1–13.

EMME, H., 1925 Beiträge zur Cytologie der Gersten. Zeitschr. indukt. Abstamm. u. Vererb. **37**: 229–236.

ERNST, A., 1922 Chromosomenzahl und Rassenbildung. Vierteljahrsch. Nat. Gesell. Zurich, **67**: 75–108.

HAASE-BESSEL, C., 1921 Digitalisstudien II. Zeitschr. indukt. Abstamm. u. Vererb. **27**: 1–26.

HOCQUETTE, M., 1922 Observations sur le nombre des chromosomes chez quelques Renonculacées. Compt. Rend. Soc. Biol. Paris **87**: 1301–1303.

JEFFREY, E. C., 1918 Evolution by hybridization. Mem. Brooklyn Bot. Gar. **1**: 298–305.

LITARDIÈRE, DE R., 1921 Recherches sur l'élément chromosomique dans la caryocinese somatique des Felicinées. Cellule **31**: 255–473.

1922 Note à propos du nombre des chromosomes chez. le *Senecio vulgaris* L. Bull. Soc. Bot. France **69**: 20–21.

LJUNGDAHL, H., 1922 Zur Zytologie der Gattung Papaver. Svensk. Bot. Tidskr. **16**: 103–114.

1924 Uber die Herkunft der in der Meiosis konjugierenden Chromosomen bei Papaver-Hybriden. Svensk. Bot. Tidskr. **18**: 277–291.

LONGLEY, A. E., 1924a Cytological studies in the genus Rubus. Amer. Jour. Bot. **11**: 249–282.

1924b Cytological studies in the genus Crataegus. Amer. Jour. Bot. **11**: 295–316.

MAGNUS, P., 1900 Eine Bemerkung zu J. Velonovsky's Mitteilung uber eine Missbildung in den Bluten des *Ranunculus acris* L. Österr. Bot. Zeitschr. **50**: 283–286.

MEYER, K., 1925 Über die Entwicklung des Pollens bei *Leontodon autumnalis* L. Bericht. Deutsch. Bot. Gesell. **43**: 108–114.

MOL, DE W. E., 1921 Over het voorkomen van heteroploide varieteiten van *Hycainthus orientalis* L. in de Hollandshe kulturen. Genetica **3**: 97–192.

NAWASCHIN, S. G., 1912 Sur le dimorphisme nucléaire des cellules somatiques de *Galtonia candicans*. Bull. Acad. Imp. Sci. Petersbourg VI, **6**: 373–385, (Russian).

1915 Les hétéro- et idiochromosomes du noyau cellulaire végétale comme cause du dimorphisme nucléaire de certaines plantes et le rôle de ce dimorphisme dans l'origine des espèces. Bull. Acad. Imp. Sci. Petersbourg VI, **9**: 1821–1834. (Russian).

NAWASCHIN, M., 1925a Morphologische Kernstudien der Crepis-Arten in Bezug auf die Artbildung. Zeitschr. Zellforsch. u. Mik. Anat. **2**: 98–111.

1925b Polyploid mutations in Crepis. Triploid and pentaploid mutants of *Crepis capillaris*. Genetics **10**: 583–592.

OVEREEM, VAN C., 1921 Über Formen mit abweichender Chromosomenzahl bei Oenothera. Beih. Bot. Centralbl. **38**: 73–113.

1923 Über Formen mit abweichender Chromosomenzahl bei Oenothera. Beih. Bot. Centralbl. **39**: 1–80.

PENZIG, O., 1922 Pflanzen-Teratologie, 2 Aufl. Bd. II.

ROSENBERG, O., 1909 Cytologische unf morphologishe Studien an *Drosera longifolia*×*D. rotundifolia*. K. Svens. Vet. Akad. Handl. **43**: 1–65.

SAKAMURA, T., 1915 Über die Einuschnurung der Chromosomen bei *Vicia Faba* L. Bot. Mag. Tokyo. **30**: 375–399.

1920 Experimentelle Studien uber die Zell- und Kernteilung mit besonderer Rucksicht auf Form, Grosse und Zahl der Chromosomen. Jour. Coll. Sci. Imper. Univ. Tokyo. **39**: 1–221.

SCHMIDT, H., 1911 Teratologische Beobachtungen an einigen einheimischen Pflanzen. Beih. Bot. Centralbl. **28**: 301.

SOROKIN, H., 1924 The satellites of the somatic mitoses in *Ranunculus acris* L. Publ. Fac. Sci. Univ. Charles, Prague **13**: 1–15.

1926 A study of the meiosis in *Ranunculus acris* L. Amer. Jour. Bot. (in press).

TAYLOR, W. R., 1924 Cytological studies on Gasteria L. Chromosome shape and individuality. Amer. Jour Bot. **11**: 51–59.

1925a The chromosome morphology of Velthemia, Allium and Cyrtanthus. Amer. Jour. Bot. **12**: 104–115.

1925b Cytological studies on Gasteria II. A comparison of the chromosomes of Gasteria, Aloe and Haworthia. Amer. Jour. Bot. **12**: 219–223.

1925c Chromosome constrictions as distinguished characteristics in plants. Amer. Jour. Bot. **12**: 238–244.

1926 Chromosome morphology in Fritillaria, Alstroemeria, Silphium, and other genera. Amer. Jour. Bot. **13**: 179–193.

TISCHLER, J., 1906 Über die Entwicklung der Sexualorgane bei einen sterilen Bryonia-Bastard. Bericht. Deutsch. Bot. Gesell. **24**: 83–96.

1921–1922 Allgemeine Pflanzenkaryologie. Linsbauers Handbuch der Pflanzenanatomie. Bd. 2. S. 897 Berlin.

TURRILL, W. B., 1919 Observations on the perianth in *Ranunculus auricomus* and *Anemone coronaria*. New Phyt. **18**: 253–256.

VELONOVSKY, J., 1900 Eine interessante Missbildung in den Blüten des *Ranunculus acris* L. Österr. Bot. Zeitschr. **50**: 244–245.

YASUI, K., 1921 On the behavior of chromosomes in the meiotic phase of some artificially raised Papaver hybrids. Bot. Mag. Tokyo, **35**: 154–168.

CHROMOSOME COUNTS IN THE VARIETIES OF *SOLANUM TUBEROSUM* AND ALLIED WILD SPECIES[1]

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As far as has been determined, the species in the *Solanaceae* have a basic haploid chromosome number of 12. BÖNICKE (1911) has shown that the haploid chromosome number in both *Datura Stramonium* L. and *Datura Tatula* L. is 12. BLAKESLEE (1921) reports tetraploid mutations in *Datura Stramonium* with the haploid chromosome number 24. BLAKESLEE and BELLING (1924) produced experimentally, by means of cold, tetraploid plants of *Datura Stramonium*. WINKLER (1910) reports that the chromosome number is 36 in the haploid cells of *Solanum nigrum* L. and 12 in the haploid cells of *Solanum lycopersicum* L. WINKLER (1916) produced tetraploids artificially in these two *Solanum* species from dehydrated callus tissue and as graft hybrids. From callus tissue dehydrated with chloral hydrate, adventitious buds arose whose cells contained the 4X chromosome number. In his grafting experiments, a tetraploid sprout was produced at the union of a wedge-shaped piece of nightshade scion with tomato stock. After union had taken place, a cut was made at the level of the union so that the tomato tissue was in the middle of the cut surface and the nightshade tissue was on both sides. All axial buds were removed to allow adventitious buds to grow on the cut surface. Periclinal and sectorial chimeras appeared and in one case a periclinal chimera proved to be a tetraploid branch. WINKLER produced a true tetraploid tomato from this branch by cutting the stem across and forcing adventitious buds from callus tissue. The cells of the tetraploid tomato branch contained 48 chromosomes. In somewhat the same way WINKLER obtained a giant nightshade plant whose cells contained 4X or 144 chromosomes. In connection with the work of BLAKESLEE and of WINKLER in the experimental production of tetraploid plants in the Solanaceae, the work of NĚMEC (1899) with potatoes is of interest. NĚMEC studied the regeneration layer which forms over cut potato tubers and reports that the chromosome number found in the cells of such tissue is surely more than 70.

[1] Papers from the Department of Biology of the MAINE AGRICULTURAL EXPERIMENT STATION, No. 177, and from the Department of Botany of the UNIVERSITY of MICHIGAN, No. 244.

The relative ease with which tetraploidy has been experimentally produced in Solanaceae would suggest that the customary vegetative reproduction of potatoes might easily, of itself, afford an opportunity for the occurrence of tetraploid mutations. By the term tetraploidy, as used in this paper, is meant the doubling of the primitive chromosome group; just how this takes place is not known at the present writing. In view of the fact that, as this paper shows, tetraploidy does occur in some of the cultivated potatoes, it is planned to attempt the artificial production of potato tetraploids from the available diploid types. Such work may give rise to the production of commercial strains of economic value. Tetraploid forms of plants are usually *gigas* forms, and *gigas* forms may possibly produce a greater yield or different quality of tubers than parental types.

It does not appear unlikely that a cytological examination of the cultivated potatoes and their wild allies may determine whether or not tetraploidy has been one of the factors in the evolution of the potato. This is the problem which is dealt with in a preliminary way in this paper. In potatoes the question of pollen sterility is a very important one. It is not impossible that one of the manifestations of sterility may be that some pollen mother cells, failing to undergo the reduction divisions produce non-functional pollen grains, whereas other microspores produced in the same anther by the normal reduction division are functional. It may also be that in certain potato varieties and species-hybrids the chromosomes are irregularly distributed to the two poles during meiosis. Although to date no study has been made of somatic material, such a study is planned for the coming season and thus evidence may be obtained for the solution of the problems suggested. It would be decidedly interesting to find out what plant breeding advances might come about through the repeated intercrossing of tetraploid plants with diploid, with the end in view of securing types with one or more reduplicated chromosomes, thus applying to a vegetatively propagated economic plant some of the results obtained by BLAKESLEE and BELLING with Datura.

The material from which this cytological study in Solanum was made was grown at Aroostook Farm of the MAINE AGRICULTURAL EXPERIMENT STATION by Doctor CHARLES F. CLARK of the Office of Horticultural Investigations, UNITED STATES DEPARTMENT OF AGRICULTURE. Doctor CLARK not only very kindly allowed the writer to have access to the material but also checked over the classification of the various forms and furnished information regarding the original sources of the wild species referred to in this paper. *S. chacoense* Bitter originally came from Paraguay, *S. demissum* Lindl. from Mexico, and *S. Fendleri* Gray and

S. Jamesii Torr. from the Alamo National Forest at Cloudcroft, New Mexico. The material was fixed in ALLEN's modification No. 15 of BOUIN's killing fluid. The cytological problem in this paper was suggested to the writer by Doctor KARL SAX of the MAINE AGRICULTURAL EXPERIMENT STATION. Doctor SAX also very kindly assisted the writer during the course of the study.

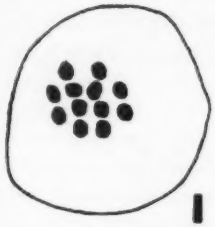


FIGURE 1.—Haploid chromosome group of *Solanum Jamesii* at heterotypic metaphase.

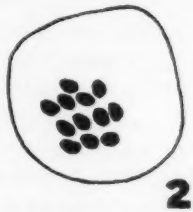


FIGURE 2.—Haploid chromosome group of *Solanum chacoense* at heterotypic metaphase.

As far as is now known the chromosome number of all the cultivated varieties of *Solanum tuberosum* L. and the allied wild species is some multiple of 12. Figures 1 and 2 illustrate that the reduced number of

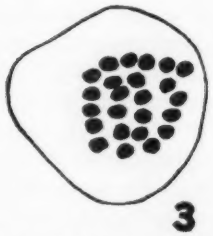


FIGURE 3.—Haploid Chromosome group of *Solanum Fendleri* at heterotypic metaphase.

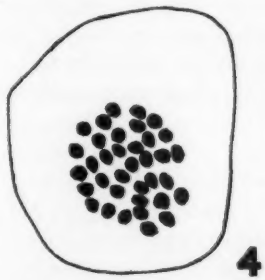


FIGURE 4.—Haploid chromosome group of *Solanum demissum* at heterotypic metaphase.

chromosomes in *Solanum Jamesii* and *Solanum chacoense* respectively is 12. The chromosomes at homotypic metaphase in haploid cells of *Solanum Jamesii* are shown also in plate 1, figure 1. For *Solanum Fendleri*, as shown in figure 3 and plate 1, figure 2, the haploid chromosome number is 24, and for *Solanum demissum* as illustrated in figure 4 and plate 1, figure 3, the haploid chromosome number is 36.

In the cultivated varieties McIntyre and McCormick, 24 is the reduced number of chromosomes. Figure 5 and plate 1, figures 4 and 5 illustrate the heterotypic metaphase as observed in pollen mother cells of the McIntyre variety. There can be no question about the count in this variety as both poles clearly show 24 chromosomes. The two equatorial

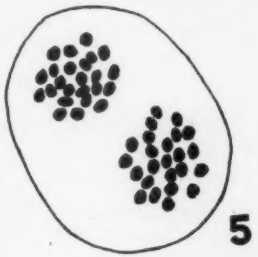


FIGURE 5.—Haploid chromosome groups of the McIntyre variety at homotypic metaphase.

plates are approximately in one plane within the cell. The same clear-cut evidence has been obtained for the McCormick variety, for which figures 6 and 7 represent the heterotypic metaphase. In this case the chromosomes at the two poles were necessarily shown in separate drawings because the two plates were in different planes, one directly beneath the other.

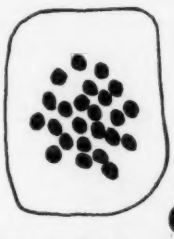


FIGURE 6.—Haploid chromosome group of the McCormick Variety at homotypic metaphase.

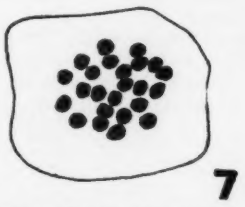


FIGURE 7.—Haploid chromosome group of the McCormick variety at homotypic metaphase.

It is believed that tetraploidy occurs in the Early Ohio variety. Figures 8 and 9 and plate 1, figures 6 and 7 present evidence for this belief. These figures illustrate a pollen mother cell of the Early Ohio variety at heterotypic metaphase. Since both poles of the division figure show unquestionably more than 36 and approximately 48 chromosomes, there can be little doubt that these figures indicate tetraploidy in this variety.

LEGEND FOR PLATE 1

FIGURE 1.—Photomicrograph showing the haploid chromosome group of *Solanum Jamesii* at heterotypic metaphase. The 12 chromosomes of this species are clearly illustrated.

FIGURE 2.—Photomicrograph showing the haploid chromosome group of *Solanum Fendleri* at heterotypic metaphase. The 24 chromosomes of this species are clearly illustrated.

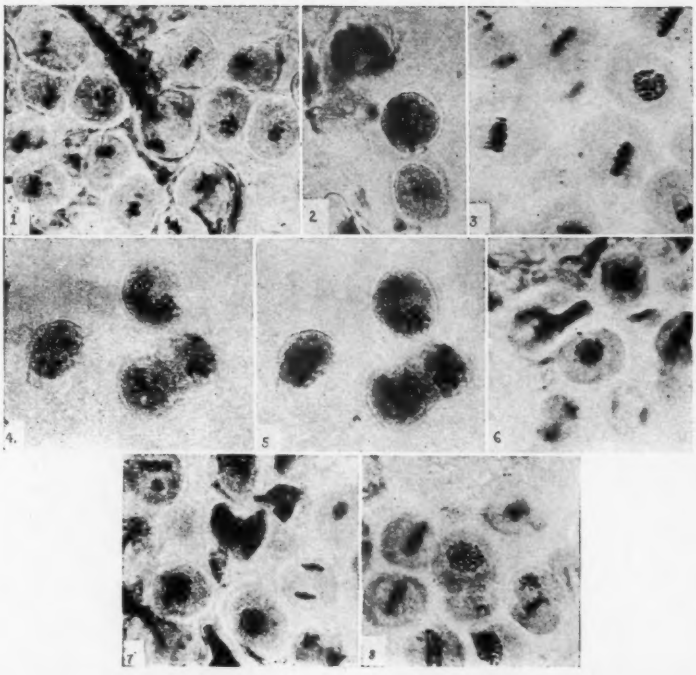
FIGURE 3.—Photomicrograph showing the haploid chromosome group of *Solanum demissum* at heterotypic metaphase. The 36 chromosomes of this species are clearly illustrated.

FIGURES 4 AND 5.—Photomicrographs of a pollen mother cell of the McIntyre variety of the cultivated potato at homotypic metaphase; in figure 4 one of the two equatorial plates is in focus and in figure 5 the other equatorial plate is in focus. These figures show that the haploid chromosome number of this variety is 24, and that the diploid chromosome number is 48.

FIGURES 6 AND 7.—Photomicrographs showing tetraploidy in the Early Ohio variety; figure 6 shows one of the two poles of the homotypic metaphase, and figure 7 shows the other pole of the same cell in focus.

FIGURE 8.—Photomicrograph showing what is believed to represent tetrapoloidy in the Early Rose variety. This figure shows the 48 chromosomes at heterotypic metaphase of the pollen mother cells.

SMITH, HUGH B. CHROMOSOME COUNTS IN *Solanum tuberosum*. PLATE 1.



At any rate the chromosome number is greater than a triploid form would have.

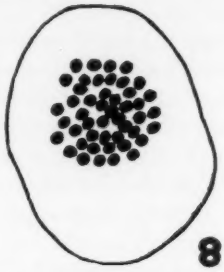


FIGURE 8.—Haploid chromosome group in the Early Ohio variety at homotypic metaphase, indicating tetraploidy in this variety.

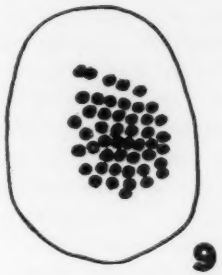


FIGURE 9.—Haploid chromosome group in the Early Ohio variety at homotypic metaphase, indicating tetraploidy in this variety.

Although the evidence is not so conclusive as that which has just been described for the McIntyre and McCormick varieties, it is, nevertheless, believed that the haploid chromosome number in the Russet Rural and Early Ohio varieties is 24, and that tetraploidy, similar to that in Early Ohio, occurs in the Early Rose and Russet Rural varieties. The evidence

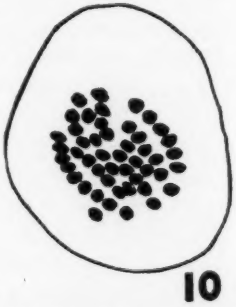


FIGURE 10.—Haploid chromosome group in the Russet Rural variety at heterotypic metaphase, indicating tetraploidy in this variety.

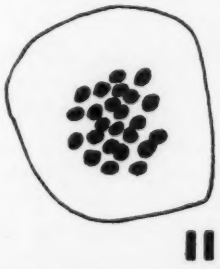


FIGURE 11.—Haploid chromosome group in the Russet Rural Variety at heterotypic metaphase.

for these tentative conclusions is presented in the following illustrations of heterotypic metaphases. Figures 10 and 11 illustrate respectively the haploid chromosome number of tetraploid and diploid plants of the Russet Rural variety. Figure 12 illustrates the haploid chromosome

number of diploid Early Ohio plants, and figures 13 and plate 1, figure 8 illustrate a pollen mother cell of the Early Rose variety at homotypic metaphase and are believed to indicate tetraploidy in this variety.

LUTMAN (1925) reports for the Irish Cobbler, Green Mountain, Lookout Mountain, and Early Rose varieties that the diploid chromosome number, as determined from a study of root tip material, is 36. This does not mean that heterotypic figures would necessarily show less than 24 chromosomes, as 12 may be single chromosomes and 12 of them paired. If this were the case, one would never be able to find 24 chromosomes at both poles of the heterotypic metaphase in these four varieties, and no such counts have been reported.

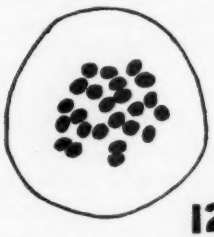


FIGURE 12.—Haploid chromosome group in the Early Ohio variety at heterotypic metaphase.

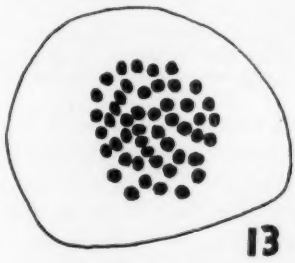


FIGURE 13.—Haploid chromosome group in the Early Rose variety at heterotypic metaphase, indicating tetraploidy in this variety.

If certain potato varieties are triploid, as is suggested by a count of 36 in somatic cells, one might find in this fact an explanation of at least part of the pollen sterility in the potato. Such a cause of sterility has been shown for Oenothera by GATES (1915), for Datura by BLAKESLEE, BELLING, and FARNUM (1920), for wheat by SAX (1921, 1922, 1922), and for roses by BLACKBURN AND HARRISON (1921). BLAKESLEE, BELLING, and FARNUM have shown that Datura mutants have a relatively high degree of pollen sterility and that the tetraploid form of *Datura Stramonium* is sterile with its parent stock. GATES states that only 11 percent of the pollen grains are viable in *Oenothera gigas* × *lata rubricalyx*. The irregular, 5- and 6-lobed grains, together with many other misshapen and sterile grains, may contain chromosome combinations which are incompatible with development. BLACKBURN AND HARRISON in their work with roses have found that univalents split at heterotypic metaphase and in many cases fail to reach the poles but form supernumerary micronuclei. Thus octads rather than tetrads are often formed and those pollen grains derived chiefly from micronuclei collapse. Such behavior is believed to

be due to hybridity. SAX working with wheat has found that the homologous chromosomes of the bivalents in species hybrids separate and pass regularly to the two poles, and univalents, if present, are distributed irregularly to one pole or the other during meiosis. He accounts for sterility in such hybrids on a hypothesis involving, "(1) the numerical and unbalanced relations of the chromosomes resulting from irregular meiotic divisions and (2) the specific interrelations of the parental chromosomes." He believes that the gametes exhibit more and more perfection as their chromosome constitution approaches more and more closely to that of the parental forms.

It has been shown by STOUT and CLARK (1924) that certain of the cultivated potato varieties have more pollen sterility than others. Perhaps the most sterile varieties have 12 univalent chromosomes and have a somatic chromosome number 36 and the least sterile ones have only paired chromosomes and have a diploid chromosome number of 48. The McIntyre and McCormick varieties do not have unpaired chromosomes and the haploid chromosome number in these two varieties is unquestionably 24; therefore, their 2X chromosome number is 48. It is of interest to note that STOUT and CLARK (1924) classify these two varieties as having a relatively high percentage of fertile pollen. Work is in progress to determine whether or not any of the commercial varieties exhibit lagging chromosomes during meiosis.

In this preliminary paper it has been shown that the haploid chromosome number is 12 for *S. Jamesii* and *S. chacoense*, 24 for *S. Fendleri*, and 36 for *S. demissum*. This is evidence that in these wild species of potato the haploid chromosome number is some multiple of 12, just as has been reported for other species of the Solanaceae. The McIntyre and McCormick cultivated varieties, which are characterized by a relatively high percentage of fertile pollen, have a haploid chromosome number of 24 and do not have unpaired chromosomes. Tetraploidy has occurred in the Early Ohio variety as shown by the appearance of haploid cells with approximately 48 chromosomes. The fact presented in this paper that the haploid chromosome number of potato has increased from 12 to 48 indicates that tetraploidy may have been a factor in the development of the cultivated varieties.

LITERATURE CITED

BLACKBURN, KATHLEEN B., and HARRISON, J. W. H., 1921 The status of the British rose forms as determined by their cytological behavior. Annals of Bot. 35: 160–188.

BLAKESLEE, A. F., 1921 Types of mutation and their possible significance in evolution. Amer. Nat. 55: 254–267.

BLAKESLEE, A. F., and BELLING, JOHN, 1924 Chromosomal chimeras in the Jimson weed. Science N.S. **60**: 19–20.

BLAKESLEE, A. F., BELLING, JOHN, and FARNUM, M. E., 1920 Chromosomal duplication and Mendelian phenomena in *Datura* mutants. Science N.S. **52**: 388–390.

BÖNICKE, L. V., 1911 Zur Kenntnis der Prophasen der heterotypischen Teilung einiger Pollenmutterzellen. Bericht. Deutsch. Bot. Gesell. **29**: 59–65.

GATES, R. R., 1915 The mutation factor in evolution. London, Macmillan and Co.

NĚMEC, B., 1899 Ueber Kern-und Zelltheilung bei *Solanum tuberosum*. Flora **86**: 214–227.

SAX, KARL, 1921 Sterility in wheat hybrids. I. Sterility relationships and endosperm development. Genetics **6**: 229–416.

1922 Sterility in wheat hybrids. II. Chromosome behavior in partially sterile hybrids. Genetics **7**: 513–552.

1922 Sterility in wheat hybrids. III. Endosperm development and F_2 sterility. Genetics **7**: 553–558.

STOUT, A. B., and CLARK, C. F., 1924 Sterilities of wild and cultivated potatoes with reference to breeding from seed. U. S. Dept. Agric. Bull. 1195: 1–32.

WINKLER, H., 1910 Über die Nachkommenshaft der *Solanum* Pfropfbastarde und die Chromosomenzahlen ihrer Keimzellen. Zeitschr. f. Bot. **2**: 1–38.

1916 Über die experimentalle Erzeugung von Pflanzen mit abweichenden Chromosomen zahl. Zeitschr. f. Bot. **8**: 417–531.